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Range-wide population structure of European sea bass *Dicentrarchus labrax*

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Abstract

The euryhaline European sea bass *Dicentrarchus labrax* L., inhabiting the coasts of the eastern Atlantic Ocean and Mediterranean Sea, has experienced many opportunities for differentiation throughout its large natural range. However evidence has been incompletely documented geographically and with an insufficient number of markers. Therefore its full range was sampled at 22 sites and individuals were genotyped with a suite of mapped markers, including 14 microsatellite loci (n = 536) and 46 neutral or gene-linked single nucleotide polymorphisms (SNPs; n = 644). We confirm that the Atlantic and Mediterranean basins harbor two distinct lineages. Within the Atlantic Ocean no pattern was obvious based on the microsatellite and SNP genotypes, except that a subtle difference between Southeastern and Northeastern Atlantic sea bass was attributed to limited introgression of alleles from Mediterranean origin. SNP genotypes of the Mediterranean lineage differentiated in three groups, probably under influence of geographical isolation. The Western Mediterranean group showed genetic homogeneity without evidence for outlier loci. The Adriatic group appeared as a distinct unit. The Eastern Mediterranean group showed a longitudinal gradient of genotypes and most interestingly an outlier locus linked to the somatolactin gene. Overall, the spatial pattern fits to those observed with other taxa of between basin segregation and within basin connectivity, which concurs well with the swimming capabilities of European sea bass. Evidence from a few outlier loci in this and other studies encourages further exploration of its regional connectivity and adaptive evolution.

Key words: adaptation; DNA microsatellite; marine fish; population structure; SNP; somatolactin

Running title: Genetic patterns of European sea bass

INTRODUCTION

As mutations, gene flow and selection leave distinct imprints in the genome, the patterns and dynamics of allelic variability and genomic architecture trace the demographic (neutral) and adaptive (non-neutral) changes that have shaped the evolutionary history of organisms. Marine fishes offer a challenging opportunity to partition neutral and adaptive patterns as most of them have a high gene diversity (DeWoody & Avise, 2000) and large effective population sizes (reviewed in Hauser & Carvalho, 2008). The high fecundity, which goes with it, provides a substrate for selection and local adaptation to the variable marine and coastal environment, despite potentially high gene flow (Hauser & Carvalho, 2008; Hellberg, 2009; Oleksiak, 2010). A greater understanding of patterns of local adaptation would lead to a better identification of marine population units, and could be useful for management decisions (Funk *et al*., 2012). Local patterns can be highly structured in time and space (Dannewitz *et al.*, 2005; Riccioni *et al*., 2013). A consequence of this observation is that the population delineation of marine organisms as inferred by genetic data may be more complex than assumed previously (Hauser & Carvalho, 2008). However it remains that temperate marine fishes demonstrate shallow population histories, as a consequence of the impact of the successive glacial cycles during the Quaternary Period (Grant & Bowen, 1998; Hauser & Carvalho, 2008). Hence, their population history has been affected by drift, non-equilibrium demographic patterns, and vicariant events that also promoted the creation of well-defined marine contact zones. The latter occurs when genetically differentiated populations come into secondary contact. Moreover, patterns of population structure are complicated by variable survival rates of larval and post-larval stages, and hence large fluctuations in cohort size (Cushing, 1990; Hjort, 1914). This sweepstake recruitment *sensu* Hedgecock (1994) may substantially influence how marine populations evolve, the structure of their coalescent, and the way neutral and non-neutral processes are inferred (see Hedgecock & Pudovkin, 2011). Overall, the marine realm requires more careful examination of the observed patterns of gene diversity, demanding an approach with a right balance between the number of loci and sample size. This should allow for a better estimate of the (outlier) markers involved in adaptive patterns (Nielsen *et al*., 2012; Teacher *et al*., 2013) by enhancing 'neutral parametrization' (i.e. low probability to detect false positive outliers; Lotterhos & Whitlock, 2014).

Among the biologically well studied marine fish taxa features the European sea bass *Dicentrarchus labrax* L. (Moronidae, Teleostei), which inhabits the continental shelves of the Northeast Atlantic Ocean and Mediterranean Sea (Pérez-Ruzafa & Marco, 2014). Wild catches in the Atlantic Ocean and Mediterranean Sea reach about 9,000 tonnes annually (FAO, 2014) while aquaculture production reaches 153,182 tonnes (FA0, 2014). Catches are not regulated, apart from a few national plans, because of incomplete data compilation, especially from recreational fishing mortality (ICES, 2004).

Lately, biomass of wild Atlantic stocks has been declining rapidly while fishing mortality has been increasing leading to a recommendation for a 80% reduced effort by ICES (2014) and the closure of the pelagic trawl fishery in January 2015.

There is clear evidence for the presence of an Atlantic and a Mediterranean lineage (Coscia *et al*., 2012; Lemaire, Versini & Bonhomme, 2005; Naciri *et al.*, 1999; Quéré *et al.*, 2012; Tine *et al.*, 2014), with a contact zone at the Almeria-Oran front (AOF). Unlike evidence from a short cyt *b* fragment (Lemaire, Versini & Bonhomme, 2005) mitochondrial diversity based on a 6383 bp fragment is higher in the Atlantic Ocean compared to the Mediterranean Sea (π = 0.00878 and 0.00352 respectively) (Rondon, 2011). Divergence time between the Western Mediterranean and Atlantic lineages has been estimated at ca. 270,000 years BP with secondary contact at the beginning of the Holocene ca. 11,500 years BP (Tine *et al.*, 2014). Populations of the Mediterranean basin are differentiated in an Eastern and a Western Mediterranean group separated along the Siculo-Tunisian Strait (Bahri-Sfar *et al.*, 2000; Cesaroni *et al.*, 1997; Quéré *et al*., 2012). Except for a large mitochondrial genetic differentiation at the AOF (F_{ST}> 0.70; (Coscia *et al*., 2012; Lemaire, Versini & Bonhomme, 2005), overall genetic differentiation is low. Genome-wide estimated nuclear differentiation was estimated at ~2.8% between the Atlantic and Western Mediterranean lineage (Tine *et al.*, 2014), and - on the basis of twenty markers - estimated to be ~2% between the two Mediterranean basins (Quéré *et al.*, 2012). Differentiation within each basin is fairly limited based on both mtDNA and nuclear markers. The Atlantic lineage shows no evidence for population expansion (Coscia *et al*., 2012) and weak evidence for genetic structure. Samples collected along the Atlantic coasts north of Portugal (Coscia & Mariani, 2011; Fritsch e*t al*., 2007; Quéré *et al*., 2010; Coscia *et al*., 2012) did not show any structure, except for the British Isles (Child, 1992) where already a genetic difference was identified at the *PGM* locus between juvenile populations from the Irish Sea and coastal UK. The western Mediterranean population is not genetically structured (Naciri *et al*., 1999; Quéré *et al*., 2012), while the Eastern Mediterranean contains more patchy populations (Bahri-Sfar *et al*., 2000; Castilho & Ciftci, 2005; Quéré *et al.,* 2012), with very limited differentiation (<2%; (Bahri-Sfar *et al*., 2000; Quéré *et al.*, 2012). Hence, similar to many other marine fishes (e.g. Milano *et al*. (2014), Ruzzante *et al*. (2006), Teacher *et al.* (2013), sea bass has a shallow nuclear genetic structure that can be largely explained by neutral demographic processes (see Patarnello, Volckaert & Castilho (2007) for a review). Nevertheless, the study of genome-wide variation in sea bass has recently demonstrated the presence of so-called genomic islands of differentiation (Tine *et al*., 2014), while Quéré *et al*. (2012) demonstrated that markers associated with genes have larger estimates of genetic differentiation both among and within basins. Such findings suggest that adaptive determinisms of genetic variation

are also present in sea bass, but hidden within the shallow structure observed for most marine fishes.

Several aspects of the genetic structure of European sea bass remain unanswered. The historical Atlantic–Mediterranean pattern is unambiguously supported by several studies (e.g. Tine *et al.* (2014), but (i) the seemingly homogenous distribution of the Atlantic and Western Mediterranean lineages has not been analyzed basin-wide with a sufficiently large number of markers (Atlantic Ocean: max. n = 13 microsatellite loci (Coscia & Mariani, 2011); Western Mediterranean Sea: max. n = 20 nuclear markers (Quéré *et al*., 2012) to conveniently partition stochastic and adaptive processes. In contrast, (ii) the observed population structure within the Eastern Mediterranean could have been overestimated because of too few markers. (iii) Some basins remain underexplored, such as the Adriatic Sea that is known to be differentiated from the Mediterranean (Garoia *et al*., 2007; Mejri *et al.*, 2011; Milana *et al.*, 2012). (iv) Finally, the information content of the nuclear microsatellite and SNP markers to detect patterns has not been fully explored (but see Quéré *et al*. (2012). Our revisit of the genetic structure of European sea bass is based on a geographically extensive sampling effort and takes advantage of a combination of nuclear putatively neutral genetic markers (existing microsatellites and newly developed SNPs) and markers putatively influenced by selection (newly developed SNPs).

MATERIALS AND METHODS

Localities and DNA extraction

The spatial sampling design covered almost the full range of European sea bass (Figure 1, Table 1). Samples from Aveiro (P), Bardawil (Eg), Fiumicino (I), Marsala (I), Muravera (I), Rabat (Mo), Tanger - Ksar es-Seghir (Mo), Thessaloniki (Gr) and Valencia (Es) have been included in one or several previous studies: Allegrucci, Caccone & Sbordoni (1999), Allegrucci, Fortunato & Sbordoni (1997), Bahri-Sfar *et al.* (2000), Bonhomme *et al*. (2002), Caccone *et al.* (1997), García de León, Chikhi & Bonhomme (1997), Lemaire *et al.* (2000), Lemaire, Versini & Bonhomme (2005), Naciri *et al.* (1999), Quéré *et al*. (2012). Samples were collected offshore except for the lagoon samples from Aveiro, Bardawil, Muravera and Venice. Our sampling strategy did not explicitly aim at a temporal analysis of population structure. A small sample of either pectoral fin or muscle tissue, or scales were stored in 80% ethanol and kept at room temperature until DNA extraction. Total genomic DNA was isolated either using the Invisorb DNA Universal Clinical HTS 96 kit or the NucleoSpin Tissue Extraction kit

(Machery-Nagel GmBH). DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific; Table 1).

Genotyping of microsatellite markers

Fourteen mapped microsatellite loci were amplified and analysed: *DLA0008*, *DLA0119*, *DLA0016*, *DLA0020*, *DLA0105*, *DLA0228E*, *DLA0244*, *DLA0237*, *DLA0248*, *DLA0146*, *DLA0142*, *DLA0110*, *DLA0145* and *DLA0140* (Chistiakov *et al*., 2005) (Table S1). All of them were selected based on their position on the linkage map in order to provide maximal coverage and level of polymorphism. Amplification of the loci was performed in a 20-µl polymerase chain reaction (PCR) cocktail containing *Taq* buffer 1x (Promega, 50 mm KCl, 10 mm Tris-HCl pH 9 at 25 °C, 0.1% TritonX-100), 1 mM $MgCl_2$, 150 nM of each primer, 70 µM dNTPs, 0.8 U of *Taq* and 50 ng of genomic DNA. Samples were amplified on a DNA Thermal Cycler (One-Advanced Euroclone) with the following thermal profile: (i) Predenaturation at 94 °C for 2 min; (ii) 30 cycles of denaturation at 94 °C for 45 s, annealing at 48 °C for 45 s, extension at 72 °C for 45 s each; (iii) additional extension at 72 °C for 10 min. The forward primers were labelled with different fluorescent dyes allowing fragment detection on an ABI PRISM 3100 or 3700 automated sequencer (with size standard ROX-400). They were combined in two multiplex assays and the fragment analysis was run at BMR Genomics (www.bmr-genomics.it). Allele scoring was performed with the software GENOTYPER v3.7 (Applied Biosystems). In order to minimize scoring errors two operators independently read and edited the program output. Only consensus genotypes (in total 98%) were retained.

Genotyping SNP markers

A total of 51 SNPs from a large data set of sequenced and *in silico* detected unique SNP candidates (Arias *et al*., 2012; Chistiakov *et al.*, 2008; Kuhl *et al*., 2010); L. Bargelloni, pers. comm.) was successfully genotyped with the MassARRAY system (Sequenom, San Diego, USA). PCR-primers and extension-primers were designed and optimized at the Genetic Service Facility of the Flanders Institute of Biotechnology (Antwerp, Belgium). Two SNPs gave no reaction in more than 25% of the individuals and were discarded from the analysis. Similarly, 26 individuals for which more than 25% of the SNPs gave no reaction were discarded and excluded from Table 1.

Out of the 49 successfully genotyped SNPs, 37 SNPs were developed from Expressed Sequence Tags (ESTs) (Table S2). Ten of them were developed by resequencing several genes in ten individuals of

Mediterranean origin (Chistiakov *et al*., 2008; D. Chistiakov, pers. com.). Twenty seven were developed by resequencing several ESTs in four individuals from the Mediterranean Sea and four individuals from the Atlantic Ocean (Arias *et al*., 2012). However 12 of these SNPs were first detected in Atlantic ESTs before being validated on Mediterranean and Atlantic individuals and 15 were discovered while resequencing the ESTs on Mediterranean and Atlantic individuals. Finally, 12 SNPs were developed from BAC end sequences and validated using Mediterranean individuals (L. Bargelloni, pers. comm.). All sequences were annotated using BLASTN and BLASTX against the GENBANK database (Table S2). In order to infer distribution across the sea bass genome, sequences were mapped to the genome of European sea bass (Kuhl *et al.*, 2010; Tine *et al*., 2014).

Genetic variation

Data quality of microsatellites was checked for null alleles, allele dropout and stuttering by using the software MICRO-CHECKER v2.2.3 (van Oosterhout *et al*., 2004). Data quality of SNPs was assessed by calculating the frequency at which the less common allele occurs in a given population (Minor Allele Frequency - MAF) and by checking Mendelian inheritance of those SNPs that were polymorphic on the Venezia-Fbis family, which has been used for mapping purposes (Chistiakov *et al.*, 2008). Loci with null alleles and non-Mendelian inheritance were discarded.

Genetic diversity was estimated for each population by calculating the mean number of alleles (A), observed and unbiased expected heterozygosities. Departure from Hardy-Weinberg expectations was assessed using Wright's inbreeding coefficient F_{IS} for each population and lineage. The significance of F_{IS} values was calculated by permutation of alleles and corrected using the sequential Bonferroni method. Significant differences between basins were tested with a non-parametric analysis of variance Kruskal-Wallis test. Linkage disequilibrium was inferred for each pair of loci within each of the 22 samples and for each pair of loci within the Atlantic and Mediterranean lineages.

Visualization of allele and genotype frequencies is done with web-based interactive geo-visualization software available at https://fishreg.jrc.ec.europa.eu/map/genetics_geobrowser (see Fig. 1). An interesting feature is that allele frequencies are plotted quantitatively. Various population genetic maps are available online for visualization and environmental data can be added as additional layers on the map.

Genetic structure

Genetic differentiation was assessed with three approaches based on all loci in order to understand overall structure before screening for outlier markers. (i) Divergence among populations was measured using Wright's pair-wise F_{ST} values for each marker type and for samples including at least 15 individuals (but see (Kalinowski, 2005)(Willing, Dreyer & van Oosterhout, 2012). The significance of F_{ST} values was calculated by permutation of individuals and corrected using the sequential Bonferroni method. All calculations were performed using the GENETIX software v4.05 (Belkhir *et al*., 1999). (ii) Individual genotypes of each marker type and the combined microsatellite and SNP markers were clustered through discriminant analysis of principal components (DAPC) (Jombart, Devillard & Balloux, 2010) as implemented in R (R Development Core Team 2014). Data was first transformed using Principal Component Analysis (PCA). After retaining an appropriate number of PCs, the k-means algorithm was run and the Bayesian Information Criterion (BIC) was used to select the most suitable *K* number of genetic clusters. As the BIC criterion is known to overestimate the number of clusters, several lower K values were tested and the value fitting the data the best was retained. Assignment of individuals to the *K* clusters and DAPC were then performed. Analyses were conducted using the ADEGENET package (Jombart & Ahmed, 2011) for the R software (http://www.r-project.org). (iii) Population structure was inferred by clustering the genotypes for all markers through running the software STRUCTURE v2.3.4 (Pritchard, Stephens & Donnelly, 2000). Unlike DAPC which maximizes genetic separation among groups and minimizes variation within groups, it groups individuals in clusters based on the minimizing of Hardy-Weinberg and linkage disequilibria. This was done for a number of populations (*K*) ranging from one to 10, using the four models available (no admixture and allele frequencies correlated, no admixture and allele frequencies not correlated, admixture and allele frequencies correlated, admixture and allele frequencies not correlated). A burn in length of 10^3 iterations and subsequently 10^4 additional Monte Carlo Markov chain (MCMC) iterations were performed. Each assessment of *K* was repeated five times to check the repeatability of the results. The most likely K, selected according to Evanno, Regnaut & Goudet (2005), was then used to assign each individual to its population.

Detection of outliers

The approach used to detect loci influenced by directional selection is based on the expectation that they exhibit lower intrapopulation variability and larger interpopulation differentiation than neutral loci (Shikano, Ramadevi & Merilä, 2010). We investigated signatures of directional selection based on three conceptually different approaches, each set in the context of an island model, to reduce the number of false positives. (i) To detect increased population differentiation, we adopted the hierarchical Bayesian method of F_{ST} (Foll & Gaggiotti, 2008). It estimates population-specific F_{ST}

coefficients accounting for different intensities of genetic drift in the various populations. We used BAYESCAN v2.1. (http://cmpg.unibe.ch/software/bayescan) to perform the analyses. A total of 10 pilot runs of 5.10^3 iterations was performed after a burn in of 50.10^3. Loci with a log10 of Bayes Factor between 1.5 and 2 and larger than 2 were considered as outlier loci with a confidence interval of 95% and 99% respectively and a q-value (i.e. minimum false discovery rate) of 0.05. (ii) In a second approach, we screened for reductions in heterozygosity between populations using the lnRH test. Indeed a reduction in heterozygosity could be caused by the occurrence of a selective sweep. LnRH tests were performed pair-wise on the microsatellite genotypes according to Kauer, Dieringer & Schlötterer (2003). LnRH estimates were standardized with a mean of 0 and a standard deviation of 1; 95 and 99 % of all loci are expected to have values ranging from -1.96 to 1.96 and -2.18 to 2.18 respectively. Loci with values outside these boundaries were considered significant. Loci were considered as outliers if they were significant in at least two pair-wise comparisons. With a false positive rate of 0.05, 25, 46, 10 and 10 false positives are expected in Atlantic, Mediterranean, Western Mediterranean and Eastern Mediterranean basins respectively. (iii) To evaluate F_{ST} and heterozygosity at the same time, we employed a coalescent approach developed by Beaumont & Nichols (1996) as implemented in Lositan (Antao *et al*., 2008). The parameters of LOSITAN were as follows: the confidence interval was set to 99 % and 99.5% with a false discovery rate set to 0.1 and 0.05 respectively, the number of permutations to 2.10^4 and the population size to 50. The infinite allele model was used for the SNP markers while the stepwise mutation model was used for microsatellite markers. In all cases, the 'neutral' mean F_{ST} was used. All three approaches have their advantages and sensitivities in detecting false negatives and positives, although BAYESCAN produces a low rate of false positives under a range of demographic scenarios (De Mita *et al*., 2013; Narum & Hess, 2011). As the detection of outliers through the independent application of multiple methods increases the certainty that these are truly non-neutral, we used the information on outliers from the tests as such (without Bonferroni correction) to minimize the number of false positives. Loci were considered under directional selection when two or three tests were significant for directional selection. Although the concept of balancing selection is well established, there are still methodological limitations for its identification in hitchhiking mapping (Hansen, Meier & Mensberg, 2010). Therefore we discuss only loci under directional or positive selection.

Patterns of geographical and genetic distance

A matrix of Euclidian distances (in m) was prepared by measuring the shortest distance between two adjacent sampling points over sea within a single basin (Atlantic Ocean and Mediterranean Sea). Geographic and genetic distances were compared through a partial Mantel test by opposing the

geographic distance matrix to the matrix of genetic distances (F_{ST} [F_{ST}/(1-F_{ST})]; (Reynolds, Weir & Cockerham, 1983) using the Mantel coefficient Z (Rousset, 1997) in the software package IBD (Bohonak, 2002). We tested isolation by geographical distance (IBD) within the Mediterranean and Atlantic basin separately because each represents an Evolutionary Significant Unit (Lemaire, Versini & Bonhomme, 2005). Following Hemmer-Hansen *et al*. (2007) we controlled geographical distance for latitude and longitude to examine if geographical distance had the same effect in each of these dimensions. In that case population structure is best explained by a pattern of isolation by geographical distance. If not, it points to a minor role for geographical distance *per se*. Low sample sizes (n < 15) relative to the high allelic diversity of the microsatellite loci were excluded; they may lead to low signal to noise ratios when calculating pair-wise F_{ST} values (Kalinowski, 2005). The significance of the Mantel test was assessed by 10^6 permutations of the population in the genetic distance matrix.

RESULTS

Genetic variation of the microsatellite and SNP loci

A total of 536 individuals from 21 sites were genotyped at 14 mapped microsatellite loci; genotyping of the samples from Thessaloniki failed (Table 1). Several sample numbers from the Mediterranean Sea were low, but all samples (in the case of DAPC and Structure analysis) and some samples (in the case of F_{ST} analysis; n > 15) were retained because of the valuable information. Seven out of 25 linkage groups (LG) are covered, with 3 loci mapping to LG 10 and 15. The average number of alleles per locus varied from 6.00 (Crete - GCRE) to 16.29 (Zeebrugge - BEL) and differed significantly between basins (p = 0.028). Variability at microsatellites seems higher in the Mediterranean sea than in the Atlantic Ocean. Microsatellite loci DLA105 and DLA248 showed distinct gradients in allele frequency between the Atlantic Ocean and Mediterranean Sea. Observed heterozygosity values ranged from 0.688 (Muravera - MUR) to 0.822 (Saint-Malo) among loci and did not differ between basins (p = 0.14). One sample (Aveiro - POR) appeared to deviate from Hardy-Weinberg equilibrium after Bonferroni correction (Table S3). Both the Atlantic Ocean and Western Mediterranean lineages showed a significant heterozygote deficiency over the 14 loci after Bonferroni correction. There was no linkage disequilibrium between all 14 microsatellite loci, which is congruent with their genomic position (Table S1).

A total of 644 individuals from 22 sites were genotyped at 49 SNP loci. All but four LGs (LG 3, 10, 19 and 21) were represented by one or more SNP(s); LG 16 and LG 17 had 4 SNPs, LG 20 had 5 SNPs. The

MAF ranged from 0.002 for locus *Dl_6k11* to 0.487 for locus *S159*. It was lower than 5% at six loci (*Dl_6k11*, *S9*, *NS35*, *S186*, *S106* and *S109*; Table S2). Observed heterozygosity values ranged between 0.205 (Wexford - IRW) and 0.304 (Muravera - MUR), and differed significantly between basins (p = 0.006; Table S4). Out of 49 successfully genotyped SNPs, 15 were monomorphic in the mapping panel and four SNPs were homozygous in the mapping parents. Loci *S110*, *S170* and *S218* were discarded from further analyses because of non-Mendelian inheritance or failed genotyping, leaving the number of utilized SNPs to 46 loci. None of the samples, except the Eastern Mediterranean ones, appeared to deviate from Hardy-Weinberg equilibrium after Bonferroni correction. Linkage disequilibrium permutation tests revealed that the 46 SNPs were independent, which is congruent with the genomic position of the SNPs (Table S2). At the lineage level, 24, 7 and 19 cases of linkage disequilibrium involving 24, 11 and 22 loci were significant after Bonferroni correction in the Atlantic Ocean, Western Mediterranean and Eastern Mediterranean respectively.

Genetic structure

Overall genetic differentiation (F_{ST}) between Mediterranean and Atlantic samples was five times lower based on microsatellites (0.057; p<0.001) (Table S5) than on SNP markers (0.295; p< 0.001) (Table S7). Multi-locus pair-wise F_{ST} among Atlantic samples varied from 0.000 to 0.018 (microsatellites) and 0.000 to 0.027 (SNPs); the samples from Rabat (MRBT) and Ksar es-Seghir (MKS1) showed above average values. Differentiation between the Eastern and Western Mediterranean Sea was small (microsatellites: 0.005; p<0.001; SNPs: 0.024; p<0.001). Within the Mediterranean basin pair-wise F_{ST} values were more variable than in the Atlantic Ocean (microsatellites: $0.001<F_{ST}<0.030$; SNPs: $0.000<F_{ST}<0.094$), especially samples in the eastern basin vary. The presence of population structure was confirmed with assignment analysis through both DAPC and STRUCTURE. DAPC identified membership of the microsatellite genotypes to three clusters, one involving Atlantic samples and two involving Mediterranean samples (Figure 2a,b). Clustering of the SNP genotypes led to four clusters, with one cluster in the Atlantic Ocean and three weakly separated clusters in the Mediterranean Sea (Figure 2c, d). Almost all Atlantic individuals were assigned to the Atlantic group while almost all Mediterranean individuals to the Mediterranean group. Individuals from the Strait of Gibraltar (Ksar es-Seghir – MKS1) clustered with the Atlantic samples. No substructure was found in the Atlantic Ocean except that three samples (Portugal - PFA, Rabat - MRBT, Ksar es-Seghir – MKS1) were influenced by Mediterranean genotypes. Mediterranean samples split in two groups: Eastern and Western Mediterranean basin (Figure 2a, b) or an additional Adriatic group in case of the SNP genotypes (Figure 2c, d). Results from the STRUCTURE analysis are

comparable to the DAPC analysis with k = 2 providing the most probable groupings including one Atlantic and one Mediterranean group (Figure S1, S2). Two groups were detected in the Atlantic basin based on the SNP markers with the samples of Faro (PFA) and Morocco (MRBT and MKS1) constituting a separate cluster (Figure 1, S3). Although the number of clusters of the SNP genotypes was evaluated to be two in the Mediterranean basin, a weak substructure of three groups was observed (Figure 1, S4).

We restricted the combined analysis of the microsatellite and SNP genotypes to DAPC, due to different assumptions underlying the analysis of the two marker types in STRUCTURE. Overall, population structure was confirmed. The scatter plot of the first two components of the DAPC fitted a hierarchical island model. The number of genetic clusters was parsimoniously estimated to be four, similar to the analysis based on SNP genotypes and one more than the microsatellite genotypes. One cluster grouped all Atlantic samples while the three others clustered all Mediterranean samples. Samples from each basin formed distinct populations, and the Mediterranean samples split in a western, Adriatic and eastern group (Figure 2e, f). Samples from Morocco (MRBT and MKS1) clustered with the Atlantic lineage while the Murcia (EMUR) sample clustered with the Mediterranean lineage. Genotypes of a few individuals from Valencia (EGLV) and Sète (FSET) clustered with the Adriatic group.

Mantel tests indicated that Atlantic samples were isolated by distance (microsatellites: Z = 196,260; r = 0.379; p = 0.04; SNPs: Z = 653598; r = 0.683; p = 0.0001). The correlation of genetic distances to geographic distances remained significant when controlling for latitude (microsatellites: r = 0.371; p = 0.04) and longitude (microsatellites: r = 0.388; p = 0.03; SNPs : r = 0.799; p = 0.003). There is a measurable effect of the southeast Atlantic samples of Rabat (MRBT) and Ksar es-Seghir (MKS1) as none of the correlations remained significant after their removal (Table S6).

Genetic and geographic distances of the Mediterranean samples (microsatellites: Z = 1,710,752; r = 0.509; p = 0.04; SNP: Z = 4,309,638; r = 0.643; p = 0.0006) and Eastern Mediterranean samples (microsatellites: Z = 283369; r = 0.899; p = 0.04; SNP: Z = 830887; r = 0.815; p = 0.02) were correlated but not the Western Mediterranean samples (microsatellites : Z = 165783; r = 0.167; p = 0.21; SNP: Z = 65774; r = -0.221; p = 0.01). None of the correlations were significant when controlling for either latitude or longitude in case of the microsatellites (Table S6).

Detection of outlier loci

The number of comparisons in which loci were detected under selection was lower than the number of false positives expected with a false positive rate of 0.05 in all lnRH tests. Global outlier tests by lineage showed weak signatures of directional selection at 2/0/9 microsatellite and 6/0 SNP loci with lnRH/LOSITAN/BAYESCAN and LOSITAN/BAYESCAN respectively in the Atlantic Ocean (Table 2). The signal was stronger in the Mediterranean lineage at 6/3/9 and 3/1 with lnRH/LOSITAN/BAYESCAN and LOSITAN/BAYESCAN respectively. Because of the sharp distinction between the two Mediterranean basins, the Mediterranean samples were analysed separately in a Western and an Eastern Mediterranean group. Here the significant outlier tests were 2/0/4 and 2/0 for the Western Mediterranean and 3/1/1 and 1/1 for the Eastern Mediterranean respectively. Since the number of comparisons in which outlier loci were detected was smaller than the number of expected false positives, those loci are probably not under directional selection. Among the SNP loci, just one locus (*SL-UTR1*; somatolactin; LG 13) was identified as a strong candidate in the Mediterranean lineage and in the Eastern Mediterranean group. Unlike elsewhere frequencies of allele *SL-UTR1-1* were higher than 0.3 in the Adriatic Sea and Northern Mediterranean Sea. As aquaculture escapees have been identified in the Bardawil (Bahri-Sfar et al., 2005) and are suspected in the Messolonghi sample (Dimitriou et al., 2007), the analysis was repeated without these samples. The LOSITAN analysis still recognized locus *SL-UTR1* as outlier (data not shown). Its allele frequencies are consistently grouped in an Eastern and Western Mediterranean area. An analysis of genetic structure without the *SL-UTR1* locus resulted in the same results as mentioned above.

DISCUSSION

In European sea bass, a set of 14 microsatellite and 46 SNP markers yielded well known and new patterns of intrapopulation genetic differentiation. As expected, there is a clear difference between the Atlantic and Mediterranean lineage. New knowledge is that the Atlantic lineage has a weak structure; it is introgressed in the southeastern range of the Atlantic Ocean by the Mediterranean lineage. Sea bass inhabiting the Mediterranean basin is structured in three groups. The western Mediterranean population is homogenously structured, while the eastern Mediterranean shows evidence of isolation by distance. Signatures of selection at two microsatellite loci and one SNP locus associated with the 3'UTR of the somatolactin gene characterize the Eastern Mediterranean group.

The power of SNP and microsatellite markers to detect historical and contemporary spatial patterns

Our study combines a set of 46 mapped and annotated SNPs with an established resource of 14 mapped anonymous microsatellite markers (Chistiakov *et al*., 2005; Chistiakov *et al*., 2008). SNPs and microsatellite markers are firmly established in population genetics, each having distinct and complementary characteristics (Hagen *et al*., 2013; Helyar *et al.*, 2011; Morin, Luikart & Wayne, 2004). With SNPs outperforming microsatellites at a finer scale in non-model organisms, greater power has been achieved to discriminate populations with SNP and microsatellite markers combined (Hess, Matala & Narum, 2011) or with genome scans based on thousands of SNP markers (Corander *et al*., 2013; Roesti *et al*., 2014). Overall, our 14 microsatellites and 46 SNPs harbor complementary information on genetic diversity and structure, with SNP observed heterozygosities being on average three times lower, estimates of F_{IS} on average comparable although not always congruent, and F_{ST} values on average several times higher between basins. Our values of genetic differentiation at the microsatellite loci (overall F_{ST} = 0.041) match published data of European sea bass (Coscia & Mariani, 2011; Fritsch *et al*., 2007; Lemaire, Versini & Bonhomme, 2005; Quéré *et al*., 2012). SNP loci show higher values (overall F_{ST} = 0.194) although three to four times lower than for mtDNA (Coscia *et al.*, 2012; Lemaire *et al*., 2000). SNP genotypes separated the North African samples from the other Atlantic samples and distinguished better between Adriatic, Western and Eastern Mediterranean samples. The combination of microsatellites and SNP genotypes provided a similar picture as the SNP genotypes, although the pattern changed somewhat in the Mediterranean Sea.

The power of markers required to discriminate among “open” marine populations is linked to their characteristics and the degree of differentiation. Increasing the number of markers from tens of microsatellites to thousands of SNPs has enhanced the resolution and confidence in individual genotypes (Novembre *et al*., 2008), although small numbers may suffice (Provan *et al*., 2013), especially in the case of SNPs (Willing, Dreyer & van Oosterhout, 2012). New genomic venues have been opened with a recent study of European sea bass based on 234,148 SNPs isolated through restriction enzyme associated DNA (RAD) genotyping (Tine *et al*., 2014).

Genetic patterns in the Atlantic Ocean

Detecting subtle genetic structure in outbred marine species with large effective population sizes requires careful analysis of neutral and non-neutral genetic variation in order to understand the balance between geographical fragmentation, connectivity and adaptation (André *et al.*, 2011;

Hemmer-Hansen *et al.*, 2013; Pujolar *et al.*, 2014). In agreement with the literature on European sea bass (see introduction) we found evidence for a sharp separation between the Mediterranean Sea and Atlantic Ocean with microsatellite and SNP markers alike. Separation has been attributed to vicariance during the Pleistocene due to changing sea levels and hydrodynamic patterns, and shifting climate zones. Genomic analysis has revealed secondary nuclear introgression from the Atlantic to the Mediterranean lineage (Tine et al., 2014) and cytoplasmic secondary introgression from the Mediterranean to the Atlantic lineage (Lemaire, Versini & Bonhomme, 2005) following re-established contact in the Holocene. The Almeria-Oran hydrodynamic front (AOF) functions as a barrier, separating cold and less saline Atlantic water from denser Mediterranean water masses. Many, although not all, marine taxa are affected by this strong environmental barrier (see review of Patarnello, Volckaert & Castilho, 2007).

So far no distinct genetic spatial pattern of the Atlantic lineage has been detected, although a large latitudinal range from 33° N (southern Morocco) to 60° N (Norway) was sampled. Southern populations did not seem to have retained an ancestral identity under influence of latitudinal range shifts during the Pleistocene. Kettle *et al.* (2011) identified in the southeastern Atlantic Ocean two refuges: the Azores, Canaries and NW Africa, and the Atlantic Iberian peninsula (Borrero-Perez *et al*., 2011; Chevolot *et al*., 2006; Roman & Palumbi, 2004; Xavier *et al*., 2011). The weak pattern judged from F_{ST} values, tests for isolation by distance and assignment analysis do not suggest vicariance but gene flow of nuclear genetic material from the Mediterranean Sea into the Atlantic Ocean (as observed at Rabat and Ksar es-Seghir. While Tine *et al.* (2014) document the introgression of the Atlantic nuclear genome into the Mediterranean, our evidence points to introgression in the opposite direction. This is in agreement with previous studies (Coscia *et al.*, 2012; Coscia & Mariani, 2011; Lemaire, Versini & Bonhomme, 2005), which identify introgression of mitochondrial genomes from the Mediterranean Sea in the Atlantic Ocean. This raises an interesting question on the permeability of the AOF system whose stability is influenced by seasonal variation in coastal currents. There is no further evidence for sea bass in the literature as unfortunately the coast of North Africa remains vastly undersampled (but see Bonhomme *et al*., 2002; Lemaire, Versini & Bonhomme, 2005; Naciri *et al*., 1999).

European sea bass has the capacity to migrate up to several hundred kilometers to the spawning grounds along the Northeastern Atlantic coasts (Fritsch *et al.*, 2007; Pawson *et al*., 2008; Pickett & Pawson, 1994). The latter authors attribute the lack of genetic differentiation to low level exchanges between populations. Also Coscia & Mariani (2011), covering sea bass populations from the Bay of Biscay up to Norway, revealed a homogenous genetic structure. However, the microsatellite-based genotypes integrate historical (Holocene) gene flow and contemporaneous exchange, and are limited

in their power to separate populations. With non-neutral markers differentiation at a finer resolution can be detected. For example, Quéré *et al*. (2010) found the somatolactin (SL) gene to differentiate the Bay of Biscay from the southern North Sea while other loci associated to candidate genes did not. Although the SL locus was included in our study, albeit genotyped with a different marker, we could not confirm the latter pattern.

The conservation implications of the weakly structured and heavily exploited sea bass populations are twofold. First, although we find no genetic differentiation, we favor the delineation of spawning stocks (Pawson, Kupschus & Pickett, 2007; Reiss *et al*., 2009). Hence, we concur with Pawson *et al*. (2007) to assign stock units in the North Sea, the Eastern English Channel, the Western English Channel, and the combined Irish and Celtic Sea. We propose three additional stocks: the Bay of Biscay following Quéré *et al*. (2010), the coasts off Portugal to Morocco and the Alboran Sea (this study). We speculate that the so far unexplored transition zone between Northwest Iberia (*Neiva et al.*, 2012) and Cape Sagres (Portugal) (Castilho & McAndrew, 1998; Martinez *et al*., 1991) might also play a role in stock delineation. As stock assessments under the guidance of ICES have only recently been introduced for the data-limited stocks of sea bass, management measures have been implemented under the precautionary rule (ICES, 2014). It is expected that with access to numerous high-resolution markers (Tine *et al*., 2014; project AQUATRACE – https://aquatrace.eu) the subtle genetic pattern of European sea bass will become better understood.

The second aspect of conservation relates to climate change and its effects on the population dynamics at the border of the distribution range. European sea bass has steadily expanded its range into northern Atlantic waters (Pawson, Kupschus & Pickett, 2007) in response to changing local conditions (Beaugrand *et al*., 2013; Cheung, Watson & Pauly, 2013; Davis & Shaw, 2001). There is also the putative disappearance of populations in the southern range, although without any firm evidence due to limited research in North African waters. Here the unique southeastern population merits close attention because global change impacts dramatically populations at the southern border of their distribution range (Provan & Maggs, 2012; Xavier *et al.*, 2011).

Genetic patterns in the Mediterranean Sea

Two main physical features have shaped the biogeography of the oligotrophic Mediterranean Sea. First, the Western and Eastern Mediterranean Sea have been influenced by different oceanographic conditions throughout the Pleistocene and Holocene era (Patarnello, Volckaert & Castilho, 2007). Geographically separate units (refuges) have led to incipient allopatric speciation as observed in the

phylogeographical and phylogenetic patterns of taxa such as the fan mussel *Pinna nobilis* (Sanna *et al*., 2013), the seagrass *Posidonia oceanica* (Arnaud-Haond *et al*., 2007) and *Pomatoschistus* gobies (Larmuseau *et al*., 2010; Mejri *et al.*, 2011). Second, water masses vary in salinity and temperature along a west to east gradient and have been influencing spatial divergence, for example in European hake *Merluccius merluccius* (Milano *et al*., 2014) and Atlantic Bluefin tuna *Thunnus thynnus* (Riccioni *et al.*, 2013). In the case of sea bass, Pleistocene cycling doesn't seem to have had that much an influence while contemporary environmental features have impacted Mediterranean populations to some degree. They are fragmented in three slightly differentiated groups, each of them associated with a subbasin. As expected, European sea bass samples split in an eastern and western group, each linked to the Eastern and Western Mediterranean basin respectively. The Siculo-Tunisian transition represents the boundary between both, similar to several other taxa such as seagrass *Posidionia* (Serra *et al.*, 2010) and the goby *Pomatoschistus tortonesei* (Mejri *et al.*, 2009). The separation of sea bass fits with previous evidence from allozyme markers (Allegrucci, Fortunato & Sbordoni, 1997) and microsatellite markers (Bahri-Sfar *et al.*, 2000; Quéré *et al*., 2012). Interestingly mitochondrial DNA polymorphism and differentiation do not differ between basins (Rondon, 2011).

European sea bass caught in the Western Mediterranean Sea show genetic homogeneity (García de León, Chikhi & Bonhomme, 1997; Lemaire, Versini & Bonhomme, 2005; Naciri *et al.*, 1999), a feature that has been attributed to hybrid swarming (Quéré *et al*., 2012). Allopatric populations of the Atlantic Ocean and Eastern Mediterranean Sea seem to have come into secondary contact in the Western Mediterranean Sea and introgressed asymmetrically (Bierne *et al*., 2011; Tine *et al*., 2014). It is obvious in the allelic profile and the genomic architecture.

The second group, inhabiting the Adriatic basin has been overlooked previously, largely because no sample from that region had been incorporated in the analyses (but see Bahri-Sfar *et al.,* 2000). The subtle differentiation shows a pattern of isolation by distance probably in response to the local physical oceanography. The Pelagosa Sill and Strait of Otranto have been determining factors in isolating the biota of the Adriatic Sea by forming a northern and central cyclonic gyre. For example, the distribution of the Mediterranean shore crab *Carcinus aestuarii* fits the current pattern and splits in three populations, each matching with a gyre (Schiavina *et al*., 2014). However, European sea bass inhabiting the Adriatic Sea did not show evidence of additional subdivision.

European sea bass shows more genetic structure in the Eastern Mediterranean Sea than in any other basin, although some small sample sizes could have influenced the outcome**.** The Messolongi fish has two genetic backgrounds, which might be attributed to escapees from local aquaculture (Dimitriou *et al*., 2007). The above average genetic distances observed by (Bahri-Sfar *et al.*, 2000; Castilho & Ciftci, 2005; Quéré *et al*., 2012) fit the isolation by distance pattern we observed. An alternative

interpretation that local populations bear the impact of a fragmented geography at low sea level stands during the Pleistocene and hence changing currents and water masses (Rohling *et al*., 2014) seems less likely. Unlike the mitochondrial haplotypes, only the microsatellite and SNP genotypes show some structure. Also, the low number of microsatellite markers used in earlier studies might have artificially inflated differentiation.

A possible role for a candidate gene: somatolactin

An interesting outcome is the geographical distribution of the outlier locus somatolactin (SL). It is increasingly appreciated in natural populations that specific genomic regions underlie variation in adaptive traits and hence may be used to delineate population units (Funk *et al*., 2012). Especially non-neutral markers, often linked to and possibly affected by local environmental conditions, have been effective in revealing subtle patterns in the ocean with its high potential for advection and animal dispersal (Cimmaruta *et al*., 2005; Nielsen *et al*., 2012; Hemmer-Hansen *et al.*, 2014). The challenge has been to link allelic variance and phenotypic change functionally, either through linkage analysis (Quantitative Trait Loci), ecological genetic approaches or combined field and lab based studies. Increasingly cases with good evidence of genes implicated have been documented (e.g., (Larmuseau *et al.*, 2009)(Jones *et al.*, 2012)(Eizaguirre *et al.*, 2012)(Williams & Oleksiak, 2011). While so far only the *EIF3E* marker gene has been identified to play a role in local adaptation of sea bass to salinity (Guinand *et al*., 2015), evidence of the growth hormone, prolactin and somatolactin genes on a regional scale (Quéré *et al*., 2010) has been criticized on the grounds of experimental bias (Guinand *et al.*, 2015). However, the somatolactin hormone appears also in our genome scan.

The hormone somatolactin is expressed in the pituitary and involved in a broad spectrum of functions including maturation, calcium regulation, body-color regulation, lipid metabolism, cortisol secretion *in vivo*, acid-base regulation, fat metabolism and background adaptation (e.g., Kaneko & Hirano (1993), Vargas-Chacoff *et al.* (2009)). It plays as significant role in the osmoregulation of sea bass (Varsamos *et al*., 2006). The architecture of the sea bass somatolactin gene (*dlSL*) includes five exons and a promoter region with a polymorphic SSR and several transcription factor binding sites (including *cis*-regulatory elements such as Pit-1a) (Quéré *et al*., 2010). SNP *SL-UTR1* genotyped in this study is located in the 3' untranslated region, 220 bp removed from the end of the coding sequence. A second SNP was found 82 bp from the first SNP. While SSR polymorphism might modulate SL expression, other elements located either upstream of the promoter or elsewhere in (non-)coding regions might also play a role. In this study, the significant differences between the Eastern and Western Mediterranean population at the somatolactin locus originate from higher values of the A

allele in the Adriatic, Ionian and Aegean Sea, which are regions of more variable salinities along the longitudinal salinity gradient. Quéré *et al.* (2012) reported a clinal pattern of genetic differentiation possibly supporting adaptive variation at one single anonymous microsatellite locus (*DLA0068*) among the twenty-one loci of sea bass studied (but see Guinand *et al.,* (2015)). The only other documented case in the Mediterranean Sea suggests that European hake carrying the $Gapdh^{120}$ and $Gpi2^{96}$ alleles might be better adapted to a salinity (Cimmaruta, Bondanelli & Nascetti, 2005). However, given the long separate histories in the Mediterranean and Atlantic basins under diverse environmental conditions sea bass have evolved to phenotypes (Gorshkov *et al*., 2004; Mylonas *et al*., 2005; Vandeputte *et al.*, 2014) with a distinct genomic architecture (Tine *et al*., 2014).

Two points should be mentioned in regards to the management of the sea bass stocks. First, the management of the commercial and recreational fishery should take into account the revealed (genetic) stock structure. While this study identified three genetically distinct stocks in the Mediterranean Sea, the eastern Mediterranean basin and the Black Sea might harbor additional diversity. Accordingly, species-specific quota and ecosystem based management should account for this by implementing at least three management units: Western Mediterranean Sea, Eastern Mediterranean Sea and Adriatic Sea.

Second, massive anthropogenic impacts on the natural populations of European sea bass raise major concerns. Expanding aquaculture in sea pens is associated with massive escapes (Arechavala-Lopez *et al*., 2011), a phenomenon also observed in other marine fish (Jørstad *et al*., 2008). Overall documentation is poor regardless of testimonies and occasional records based on ecological (Toledo-Guedes, Sanchez-Jerez & Brito, 2014) and genetic evidence (Bahri-Sfar *et al*., 2005; Katsares *et al*., 2005; Triantafyllidis, 2007). Concerns for genetic introgression are high because domesticated sea bass have a different phenotypic and genetic profile than their natural conspecifics. This is either because of the source of the stock or because of intensive selection. As a consequence reared fish may be sources of local infections (Arechavala-Lopez *et al*., 2013) and interbreeding with wild conspecifics, especially in the vicinity of the spawning grounds. We could not test for escapees because our analysis did not include reference samples from aquaculture and our genome sampling is too sparse to detect hybrids.

In conclusion, two distinct lineages of European sea bass inhabit the Atlantic Ocean and Mediterranean Sea. Within each basin isolation by distance has further shaped the spatial structure; the pattern is weak in the Atlantic Ocean and shows evidence for three divisions in the Mediterranean Sea, most likely the result of geographical isolation. Further genomic studies might resolve this standing question, together with other open questions related to the nature of

introgression in the Atlantic-Mediterranean contact zone, adaptation to longitudinal and latitudinal gradients, the relationship between lagoon and offshore populations, and the impact of fishing pressure.

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CONFLICT OF INTEREST

None of the authors declares a conflict of interest.

DATA ARCHIVING

SNP and microsatellites genotypes have been deposited in the DRYAD databank: doi:xxx.

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Figure 1 Location of samples and sample-specific genetic differentiation by basin of *Dicentrarchuslabrax* based on assignment analysis in STRUCTURE with SNP markers. (a) Atlantic Ocean; (b) Mediterranean Sea. The colors used to represent the frequencies are derived from the CMYK color model. One of each of the colors cyan, magenta, yellow and black are assigned to each of the clusters represented. Colors are mixed according to the proportions of the representative clusters at each geographic location. The graph in the bottom left hand corner of the display shows the precise proportion of each cluster at the represented location. For site locations see Table 1. For full color representation consult the e-paper.

Figure 2 DAPC-based clustering of microsatellite genotypes (a and b), SNP genotypes (c and d) and the combined microsatellite and SNP genotypes (e and f) of European sea bass. Panels a, c and e show the DAPC plot of the clusters and panels b, d and f show the results for membership probability per individual organized by sampling site. See Table 1 for codes. For full color representation consult the e-paper.

SUPPLEMENTARY FIGURES

Figure S1 STRUCTURE bar graphs for 536 individuals of European sea bass showing membership to *k*=2, 3 and 4 clusters. Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each color a cluster. For full color representation consult the e-paper.

Figure S2 STRUCTURE bar graphs for 644 individuals of European sea bass showing membership to *k*=2, 3 and 4 clusters. Each vertical bar represents an individual genotyped at 46 SNP loci, and each color a cluster. For full color representation consult the e-paper.

Figure S3 STRUCTURE bar graphs for all individuals of European sea bass from the Atlantic Ocean showing membership to *k*=2 and 3 clusters genotyped at (a) 14 microsatellite (n = 214) and (b) 46 SNP loci (n=256). Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each color a cluster. For full color representation consult the e-paper.

Figure S4 STRUCTURE bar graphs for all individuals of European sea bass from the Mediterranean Sea showing membership to *k*=2, 3 and 4 clusters genotyped at (a) 14 microsatellite (n = 322) and (b) 46 SNP loci (n = 392). Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each color a cluster. For full color representation consult the e-paper.

LIST OF TABLES

Table 1 Description of the samples of *Dicentrarchus labrax* collected, including geographic origin, sampling site, code, sampling date, sample size (*N*) of fish genotyped for SNPs, microsatellites and SNPs and microsatellites combined.

See file Souche-Table_1_Description_of_samples-20150331.doc

Table 2 SNP and microsatellite loci detected under putative directional selection by the software packages LOSITAN and BAYESCAN in Atlantic, Mediterranean, Western Mediterranean and Eastern Mediterranean samples of European sea bass. Results from the LNRH tests are shown for microsatellites as the number of combinations in which loci were detected under selection (only numbers > 2 are reported). Loci indicated by * and ** were detected with a confidence interval of 99 % and 99.5 % for LOSITAN and 95% and 99% for BAYESCAN . Results are listed as LNRH/LOSITAN/BAYESCAN for microsatellites and LOSITAN/BAYESCAN for SNPs. Outlier loci detected by at least two approaches are indicated in bold.

	Locus	Atlantic Ocean	Mediterranean Sea	Western Mediterranean Sea	Eastern Mediterranean Sea
Microsatellites	DLA0016	- / - / **	- / ** / -		
	DLA0119	**- / - / ****	**2 / * / -**		
	DLA0248		**7 / ** / -**		**2 / ** / -**
	DLA0110	4 / - / -	- / - / **		
	DLA0008	**6 / - / ****	**3 / - / ****	**- / - / ****	**2 / - / ****
	DLA0145	**- / - / ***	**3 / - / ****	**3 / - / -**	
	DLA0142	**- / - / ****	**5 / - / ****	**- / - / ****	**2 / - / -**
	DLA0146	**- / - / ****	**2 / - / ****	**2 / - / ****	
	DLA0140	- / - / *	- / - / **		
	DLA0020	- / - / **	- / - / *		
	DLA0105	- / - / **	- / - / **	- / - / **	
	DLA0228		- / - / *		
SNPs					
	Dl_21p3	* / -			
	Dl_26f8	** / -	** / -	** / -	
	Dl_32m8	** / -			
	Dl_36d23	** / -			
	Dl_38e22	** / -			
	ILB1-int2	** / -			
	SL-UTR1	**** / ****		**** / ***	
	SOX10		** / -	* / -	
	YY		* / -	** / -	

SUPPLEMENTARY INFORMATION

Table S1 Characteristics of the sequences containing microsatellites, including the name of the microsatellite, the functional annotation of the sequence, the linkage group of the sequence in the sea bass genome, the repeat motif, the number of alleles and the GenBank accession number

Microsatellite name	Sequence annotation	Sea bass linkage group	Repeat motif	N alleles	GenBank accession number
DLA0008	-	LG 24	(ac)24	57	AY221746
DLA0016	-	LG 1	(tg)24	28	AY262073
DLA0020	-	LG 12	(tg)20	19	AY262077
DLA0105	-	LG 8	(ac)16	28	AY262082
DLA0110	-	LG 25	(gt)17	22	AY262087
DLA0119	-	LG 14	(tg)10	26	AY302259
DLA0140	-	LG 15	(ac)30	23	AY430370
DLA0142	Ephrin receptor fragment	LG 15	(ac)30	31	AY430372
DLA0145	-	LG 17	(tc)20	22	AY430375
DLA0146	-	LG 10	(tg)27	38	AY430376
DLA0228E	-	LG 10	(aaag)3(ag)4(aaag)3	14	AY639896
DLA0237PY	mRNAfor Peptide Y	LG 1	(tc)4(c)2(tc)2(c)2(tc)10	9	AJ005380
DLA0244	-	LG 10	(tg)12(ag)5(tg)2	11	AY714328
DLA0248	-	LG 15	(tc)5(ac)(at)(tc)5(t)2(tc)7(ac)3(acgc)4	9	AY714332

Table S2 Characterisation of the sequences containing SNPs by the procedure of development, including the name of the SNP, the functional annotation of the sequence, the SNP annotation (S: synonymous; NS: non-synonymous), the location of the sequence in the sea bass genome (linkage group number), the frequency of the minor allele (MAF) and the GenBank accession number. Loci that were discarded from the analysis because of non-Mendelian inheritance or putative presence of null alleles are indicated by *

SNP development	SNP name	Sequence annotation	Sea bass linkage group	SNP location	MAF	GenBank accession number
ESTs, Atlantic samples	S25	-	LG13	-	0.226	JM497213
	S29	cathepsin C precursor	LG13	-	0.364	JM497214
	S30	cornifelin	LG16	-	0.105	JM497216
	S66	stress-induced-phosphoprotein 1	LG14	CDS (S)	0.432	JM497229
	S79	-	LG5	-	0.167	JM497236
	S98	-	LGx	-	0.204	JM497249
	S106	Ferritin--livermiddlesubunit	LG8	CDS (S)	0.025	JM497160
	S110*	ATP synthase -- H+ transporting -- mitochondrial Fo complex -- subunit B1	LG22-25	CDS (S)	0.196	JM497164
	S115	MOB1 -- Mps One Binder kinase activator-like 2A	LG4	CDS (S)	0.469	JM497167
	S118	zincfinger, DHHC-type containing 4	LG1B	intronic	0.333	JM497168
	S131	UPF0561 protein C2orf68 homolog	LG20	?	0.274	JM497176
	S159	glutathione S-transferasealpha 5	LG11	CDS (S)	0.487	JM497181
ESTs, Mediterranean and Atlantic samples	S9	Hemoglobin subunit beta-1 (Hemoglobin beta-1 chain)(Beta-1-globin)(Beta-A1-globin)	SB	intronic	0.004	JM497243
	S78	potassium intermediate/small conductance calcium-activated channel -- subfamily N -- member 4	LG16	?	0.173	JM497235
	S89	RCD1 required for cell differentiation1 homolog (S. Pombe)	LG15	intronic	0.134	JM497242
	S90	Macrophage mannose receptor 1	LG9	intronic	0.442	JM497244
	S91	SUB1 homolog (S. Cerevisiae)	LG20	intronic	0.061	JM497245
	S109	Obg-likeATPase 1 (EC 3.6.3.-)	LG12	CDS (S)	0.043	JM497162
	S119	Sin3A-associated protein -- 18kDa	LG24	CDS (S)	0.208	JM497169
	S148	Uncharacterizedprotein C8orf59 homolog	LG18	UTR	0.052	JM497180
	S170*	Uncharacterizedprotein	LG1A	CDS	0.272	JM497186
	S181	superoxide dismutase 1 – soluble	LG14	UTR	0.165	JM497190
	S186	protein tyrosine phosphatase -- non-receptor type 9	LG6	intronic	0.013	JM497192
	S218*	Hemoglobin subunit alpha (Hemoglobin alpha chain)(Alpha-globin aa1)	SB	CDS (S)	0.215	JM497211
	NS13	-	LG20	-	0.210	JM497135
	NS35	fructosamine 3 kinase	LG8	intronic	0.005	JM497148
	NS36	Keratin--type I cytoskeletal 18	LG22-25	UTR	0.247	JM497149
ESTs, Mediterranea	SL-UTR1	somatolactin	LG13	UTR	0.141	AJ277390
	ERB-UTR	estrogen receptor 2 (ER beta)	LG17	UTR	0.294	AJ489523

n samples	MT-int1	Metallothionein (MT)	LG6	CDS (NS)	0.403	AF199014
	RAG-ex2	recombinationactivating gene 1	LG6	intronic	0.451	AF369066
	ACB-UTR	Beta-actin (Fragment)	LG8	UTR	0.168	AJ537421
	SOX10	SRY (sex determining region Y)-box 10	LG7	intronic	0.202	AY247003
	NPY-UTR	neuropeptide Y	LG9	UTR	0.096	AJ005378
	ILB1-int2	interleukin 1 -- beta	LG20	intronic	0.385	AJ311925
	CYP1	cytochrome P450 -- family 1 -- subfamily A -- polypeptide 2	LG5	UTR	0.295	AJ251913
	YY	Peptide YY-like	LG16	CDS (S)	0.202	AJ005379
BAC end sequences, Mediterranean samples	Dl_4h3	-	LG9	-	0.151	
	Dl_13f21	LIM domain binding 2	LG2	intronic	0.119	
	Dl_21p3	-	LG6	-	0.331	
	Dl_13o18	-	LG17	-	0.179	
	Dl_9f6	phospholipase B1	LG12	?	0.338	
	Dl_26f8	ankyrin repeat and SOCS box containing 5	LG2	intronic	0.440	
	Dl_32m8	-	LG7	-	0.479	
	Dl_22l7	-	LG20	-	0.196	
	Dl_36d23	ADAM metallopeptidase with thrombospondin type 1 motif -- 16	LG16	intronic	0.410	
	Dl_6k11	protein tyrosine phosphatase -- receptor type -- S	LG4	intronic	0.002	
	Dl_37g3	gem (nuclear organelle) associated protein 5	LG14	intronic	0.338	
	Dl_38e22	O-6-methylguanine-DNA methyltransferase	LG11	intronic	0.421	

Table S3 Genetic variability and multi-locus F_{IS} estimates at 14 microsatellites of *Dicentrarchus labrax*. A = Average number of alleles per locus; H_{exp} = unbiased expected heterozygosity; H_{obs} = observed heterozygosity; F_{IS} = multi-locus F_{IS} estimate (significant values are listed before and after Bonferroni correction underlined and in bold respectively)

Sample	A	H_{exp}	H_{obs}	F_{IS}
NOR	15.00	0.807 (0.17)	0.788 (0.19)	0.023
IRW	10.93	0.804 (0.18)	0.786 (0.18)	0.023
IRC	12.57	0.815 (0.17)	0.793 (0.18)	0.028
BEL	16.29	0.819 (0.16)	0.817 (0.17)	0.003
FSML	11.64	0.790 (0.18)	0.822 (0.17)	-0.043
POR	11.14	0.789 (0.19)	0.701 (0.14)	**0.115**
PFA	9.36	0.825 (0.13)	0.786 (0.18)	0.050
MRBT	12.00	0.793 (0.17)	0.732 (0.18)	0.079
MKS1	11.86	0.822 (0.13)	0.783 (0.13)	0.049
EMUR	13.29	0.780 (0.14)	0.780 (0.13)	-0.001
EGLV	9.21	0.765 (0.15)	0.707 (0.13)	0.079
FSET	13.07	0.794 (0.13)	0.784 (0.13)	0.014
MUR	10.43	0.767 (0.15)	0.688 (0.16)	0.110
FCT	7.71	0.755 (0.18)	0.755 (0.17)	0.000
SCL	9.36	0.799 (0.13)	0.766 (0.19)	0.042
IVEN	11.64	0.773 (0.14)	0.760 (0.11)	0.016
IPT	11.57	0.763 (0.13)	0.738 (0.09)	0.033
CMT	8.21	0.775 (0.14)	0.821 (0.20)	-0.063
GMES	10.79	0.762 (0.15)	0.769 (0.14)	-0.009
GCRE	6.00	0.768 (0.14)	0.818 (0.14)	-0.068
EGL	7.50	0.717 (0.21)	0.707 (0.20)	0.014
Atlantic Ocean	22.07	0.814 (0.16)	0.785 (0.14)	**0.036**
Mediterr. Sea	19.93	0.781 (0.14)	0.757 (0.10)	0.016
West Mediterr.	18.50	0.787 (0.14)	0.756 (0.11)	**0.04**
East Mediterr.	16.43	0.773 (0.14)	0.758 (0.11)	0.02

Table S4 Genetic variability and multi-locus F_{IS} estimates at 46 SNPs of *Dicentrarchus labrax*. A = Average number of alleles per locus; H_{exp} = unbiased expected heterozygosity; H_{obs} = observed heterozygosity; F_{IS} = multi-locus F_{IS} estimate. Significant values before and after Bonferroni correction are listed underlined and in bold respectively

Sample	A	H_{exp}	H_{obs}	F_{IS}
NOR	1.80	0.221 (0.20)	0.227 (0.21)	-0.029
IRW	1.76	0.229 (0.21)	0.205 (0.19)	0.106
IRC	1.72	0.216 (0.21)	0.205 (0.21)	0.052
BEL	1.83	0.224 (0.20)	0.219 (0.20)	0.024
FSML	1.87	0.224 (0.19)	0.228 (0.20)	-0.020
POR	1.72	0.221 (0.19)	0.236 (0.22)	-0.071
PFA	1.83	0.270 (0.19)	0.279 (0.22)	-0.038
MRBT	1.87	0.255 (0.18)	0.257 (0.19)	-0.009
MKS1	1.87	0.248 (0.19)	0.231 (0.18)	0.072
EMUR	1.96	0.288 (0.17)	0.287 (0.18)	0.003
EGLV	1.93	0.288 (0.17)	0.261 (0.16)	0.093
FSET	1.89	0.292 (0.18)	0.290 (0.18)	0.009
MUR	1.87	0.289 (0.17)	0.304 (0.19)	-0.056
FCT	1.87	0.288 (0.17)	0.278 (0.19)	0.037
SCL	1.87	0.292 (0.17)	0.290 (0.18)	0.006
IVEN	1.91	0.272 (0.19)	0.263 (0.18)	0.034
IPT	1.91	0.283 (0.18)	0.268 (0.18)	0.056
CMT	1.91	0.324 (0.18)	0.283 (0.18)	0.132
GMES	1.85	0.283 (0.18)	0.286 (0.19)	-0.010
GCRE	1.67	0.254 (0.21)	0.273 (0.26)	-0.082
GTSK	1.89	0.239 (0.17)	0.235 (0.19)	0.019
EGL	1.70	0.217 (0.20)	0.204 (0.19)	0.062
Atlantic Ocean	1.98	0.233 (0.18)	0.229 (0.18)	0.01
Mediterr. Sea	1.98	0.287 (0.17)	0.272 (0.16)	0.023
West Mediterr.	1.98	0.290 (0.17)	0.285 (0.16)	0.02
East Mediterr.	1.93	0.278 (0.18)	0.259 (0.17)	**0.06**

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Table S5 Matrix of pair-wise estimates of F_{ST} (Θ) using all 14 microsatellites of *Dicentrarchus labrax* and sample sites with at least 15 individuals. Significant values before and after sequential Bonferroni correction are indicated underlined and bold respectively. Atlantic and Alboran samples are indicated in italics. For sample codes see Table 1

	IRW	*IRC*	*BEL*	*FSML*	*POR*	*MRBT*	*MKS1*	EMUR	EGLV	FSET	MUR	FCT	SCL	IVEN	IPT	GMES
NOR	0.	0.	0.	0.	0.0037	0.0121	0.0055	**0.0607**	**0.0738**	**0.0622**	**0.0663**	**0.0791**	**0.0559**	**0.0773**	**0.0809**	**0.0865**
IRW		0.	0.	0.	0.0009	0.0134	0.0030	**0.0548**	**0.0609**	**0.0594**	**0.0608**	**0.0705**	**0.0502**	**0.0728**	**0.0737**	**0.0841**
IRC			0.0013	0.0022	0.0044	0.0182	0.0035	**0.0564**	**0.0666**	**0.0579**	**0.0629**	**0.0807**	**0.0481**	**0.0723**	**0.0739**	**0.0807**
BEL				0.0014	0.0064	0.0093	0.0076	**0.0439**	**0.0552**	**0.0451**	**0.0508**	**0.0595**	**0.0385**	**0.056**	**0.0621**	**0.068**
FSML					0.0017	0.0124	0.0043	**0.0696**	**0.0809**	**0.066**	**0.0749**	**0.0937**	**0.0651**	**0.084**	**0.089**	**0.0989**
POR						0.0084	0.0024	**0.0688**	**0.0737**	**0.0642**	**0.0698**	**0.086**	**0.0562**	**0.0763**	**0.0827**	**0.0908**
MRBT							**0.0172**	**0.0561**	**0.0666**	**0.0587**	**0.0576**	**0.0712**	**0.0492**	**0.0621**	**0.0763**	**0.0847**
MKS1								**0.0485**	**0.0486**	**0.0457**	**0.0457**	**0.0646**	**0.0372**	**0.0582**	**0.0547**	**0.0676**
EMUR									**0.017**	0.0009	0.0048	**0.0208**	**0.0152**	**0.0127**	**0.0125**	0.01
EGLV										0.0165	0.0039	0.0245	0.0106	**0.016**	0.0068	0.0161
FSET											0.0065	**0.0297**	**0.0206**	**0.0148**	**0.0179**	**0.0137**
MUR												0.0094	0.0039	0.0082	0.0049	0.0085
FCT													0.0187	**0.0255**	**0.0191**	**0.0295**
SCL														0.0111	0.0113	**0.0202**
IVEN															**0.011**	**0.0154**
IPT																**0.0101**

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Table S6 Results of the Mantel tests for evidence of isolation by distance for microsatellite and SNP loci of European sea bass, including partial Mantel tests when controlling for latitude, longitude, geographic and genetic distances. *Z*: Mantel coefficient; *r*: correlation index; *p*: significance level (significant values are listed in bold)

			Atlantic	Mediterranean	Western Mediterranean	Eastern Mediterranean
Microsatellites	Genetic distances vs Geographic distances	*Z*	196,260	1,710,752	165,783	283,369
		r	0.379	0.509	0.167	0.899
		p	**0.038**	**0.042**	0.211	**0.039**
	Genetic distances vs Geographic distances, controlling for latitude	*r*	0.371	0.264	0.174	0.866
		p	**0.045**	0.052	0.207	0.204
	Genetic distances vs Geographic distances, controlling for longitude	*r*	0.388	0.047	-0.310	0.494
		p	**0.025**	0.370	0.233	0.332
	Genetic distances vs latitude	*Z*	1.718	5.621	0.570	1.538
		r	0.559	0.561	0.071	0.839
		p	**0.002**	0.057	0.408	**0.042**
	Genetic distances vs latitude, controlling for genetic distances	*r*	0.555	0.373	0.086	-0.782
		p	**0.036**	0.063	0.389	0.204
	Genetic distances vs longitude	*Z*	0.474	13.464	1.616	2.241
		r	-0.119	0.646	0.303	0.892
		p	0.405	**0.029**	0.084	**0.039**
	Genetic distances vs longitude, controlling for genetic distances	*r*	0.150	0.463	0.395	-0.439
		p	0.155	**0.037**	0.149	0.332
SNPs	Genetic distances vs	*Z*	653,598	4,309,638	65,775	830,888

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Geographic distances	*r*	0.683	0.643	-0.221	0.815
	p	**0.000**	**0.001**	0.143	**0.017**
Genetic distances vs Geographic distances, controlling for latitude	*r*	-0.156	0.536	-0.226	0.713
	p	0.246	**0.001**	0.147	**0.034**
Genetic distances vs Geographic distances, controlling for longitude	*r*	0.799	0.541	-0.128	0.478
	p	**0.003**	**0.010**	0.409	0.115
Genetic distances vs latitude	*Z*	4.995	12.130	0.251	3.839
	r	0.736	0.485	-0.043	0.603
	p	**0.001**	**0.015**	0.463	**0.048**
Genetic distances vs latitude, controlling for genetic distances	*r*	0.405	0.265	-0.064	-0.264
	p	**0.018**	0.114	0.443	0.271
Genetic distances vs longitude	*Z*	1.615	29.477	0.599	5.806
	r	-0.075	0.432	-0.188	0.751
	p	0.465	**0.028**	0.147	**0.017**
Genetic distances vs longitude, controlling for genetic distances	*r*	-0.571	-0.136	0.045	-0.040
	p	**0.018**	0.265	0.472	0.481

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Table S7 Matrix of pair-wise estimates of F_{ST} (Θ) using all the 46 SNPs of *Dicentrarchus labrax* and sample sites with at least 15 individuals. Significant values before and after sequential Bonferroni correction are indicated underlined and bold respectively. Atlantic and Alboran samples are indicated in italics. For sample codes see Table 1

	IRW	*IRC*	*BEL*	*FSML*	*POR*	*MRBT*	*MKS*	EMUR	EGLV	FSET	MUR	FCT	SCL	IVEN	IPT	GMES	GTSK	EGL
NOR	0.	0.0036	0.0047	0.	0.0057	**0.021**	**0.0271**	**0.2753**	**0.2944**	**0.3138**	**0.3123**	**0.2842**	**0.3003**	**0.3543**	**0.385**	**0.3617**	**0.4297**	**0.4443**
IRW		0.	0.	0.	0.0031	0.0213	0.0228	**0.2626**	**0.2824**	**0.3005**	**0.2942**	**0.2709**	**0.2849**	**0.3379**	**0.371**	**0.3499**	**0.4216**	**0.4391**
IRC			0.0024	0.0067	0.0022	0.0223	0.0211	**0.2636**	**0.2818**	**0.2988**	**0.2951**	**0.273**	**0.2874**	**0.3377**	**0.3707**	**0.3489**	**0.4206**	**0.4369**
BEL				0.0024	0.0014	**0.0265**	**0.0236**	**0.2765**	**0.2966**	**0.3157**	**0.3109**	**0.2837**	**0.302**	**0.351**	**0.3846**	**0.3627**	**0.4298**	**0.4415**
FSML					0.0045	0.0143	**0.026**	**0.2654**	**0.287**	**0.3064**	**0.3007**	**0.2769**	**0.2962**	**0.342**	**0.3776**	**0.3539**	**0.4173**	**0.4281**
POR						0.0154	0.0096	**0.2424**	**0.2627**	**0.2818**	**0.2769**	**0.2499**	**0.2713**	**0.3247**	**0.3568**	**0.3319**	**0.409**	**0.421**
MRBT							0.0087	**0.1699**	**0.1881**	**0.2116**	**0.2059**	**0.1753**	**0.1968**	**0.2457**	**0.2855**	**0.2585**	**0.3187**	**0.3304**
MKS1								**0.1903**	**0.2034**	**0.2242**	**0.2204**	**0.1982**	**0.2113**	**0.2675**	**0.2999**	**0.2761**	**0.345**	**0.3595**
EMUR									0.0007	0.0044	0.0039	0.	0.0172	**0.0346**	**0.0723**	**0.0327**	**0.0647**	**0.0794**
EGLV										0.	0.0015	0.0008	0.0048	**0.0209**	**0.0481**	**0.0183**	**0.0456**	**0.0566**
FSET											0.0009	0.0072	0.0127	**0.0206**	**0.0438**	**0.0166**	**0.0429**	**0.0479**
MUR												0.0088	0.0125	**0.0286**	**0.0648**	**0.0182**	**0.0434**	**0.0656**
FCT													0.0193	**0.0284**	**0.0561**	**0.0329**	**0.0761**	**0.0889**
SCL														**0.0409**	**0.068**	**0.0383**	**0.0699**	**0.0944**
IVEN															0.0173	**0.0242**	**0.05**	**0.0519**
IPT																**0.0403**	**0.0744**	**0.0758**
GMES																	0.014	**0.0519**
GTSK																		**0.0403**

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END

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Range-wide population structure of European sea bass *Dicentrarchus labrax*

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Abstract

The euryhaline European sea bass *Dicentrarchus labrax* L., inhabiting the coasts of the eastern Atlantic Ocean and Mediterranean Sea, has experienced many opportunities for differentiation throughout its large natural range. However evidence has been incompletely documented geographically and with an insufficient number of markers. Therefore its full range was sampled at 22 sites and individuals were genotyped with a suite of mapped markers, including 14 microsatellite loci (n = 536) and 46 neutral or gene-linked single nucleotide polymorphisms (SNPs; n = 644). We confirm that the Atlantic and Mediterranean basins harbor two distinct lineages. Within the Atlantic Ocean no ~~a consistent~~ pattern ~~of isolation by distance~~ was obvious~~served~~ ~~for~~ based on the microsatellite and SNP genotypes, except that~~. Additionally,~~ a subtle difference between Southeastern and Northeastern Atlantic sea bass w~~might be~~as attributed to limited introgression of alleles from Mediterranean origin. SNP genotypes of the Mediterranean lineage differentiated in three groups, probably under influence of ~~recent~~ geographical isolation. The Western Mediterranean group showed genetic homogeneity without evidence for outlier loci. T~~; t~~he Adriatic group appeared as a distinct unit. The Eastern Mediterranean group showed a longitudinal gradient of genotypes and most interestingly ~~three statistically significant~~an outlier locus linked to the~~i~~ ~~two neutral microsatellite markers and the~~ somatolactin gene. Overall, the spatial pattern fits to those observed with other taxa of between basin segregation and within basin connectivity, which concurs well with the swimming capabilities of European sea bass. Evidence from a few outlier loci in this and other studies encourages further exploration of its regional connectivity and adaptive evolution.

Key words: adaptation; DNA microsatellite; marine fish; population structure; SNP; somatolactin

Running title: Genetic patterns of European sea bass

INTRODUCTION

As mutations, gene flow and selection leave distinct imprints in the genome, the patterns and dynamics of allelic variability and genomic architecture trace the demographic (neutral) and adaptive (non-neutral) changes that have shaped the evolutionary history of organisms. Marine fishes offer a challenging opportunity to partition neutral and adaptive patterns as~~, for~~ most of them~~, they are~~ hav~~eing~~ a high gene diversity (DeWoody & Avise, 2000) and large effective population sizes ~~even though the ratio of effective and census population size is often very low~~ (reviewed in ~~(~~Hauser & Carvalho, 2008). The high fecundity, which goes with it, provides a substrate for selection and local adaptation to the variable marine and coastal environment, despite potentially high gene flow (Hauser & Carvalho, 2008; Hellberg, 2009; Oleksiak, 2010). A greater understanding ~~in~~ of patterns of local adaptation would lead to a better identification of marine population units, and could be useful for management decisions (Funk *et al.*, 2012). ~~Indeed, studies have proved that subtle, shallow genetic patterns can be biologically meaningful and relevant (Knutsen *et al.*, 2011; Ruzzante *et al.*, 2006; Teacher *et al.*, 2013).~~ Local patterns can be highly structured in time and space~~. For example the spatially panmictic European eel Anguilla Anguilla shows temporally structured genetic differentiation (Baltazar-Soares et al., 2014) and spawning populations of Atlantic bluefin tuna Thunnus thynnus in the Mediterranean Sea are affected by the local physical oceanography~~ ~~(~~(Dannewitz *et al.*, 2005; Riccioni *et al.*, 2013)~~. Riccioni et al., 2013)~~. A consequence of this observation is that the population delineation of marine organisms as inferred by genetic data may be more complex than assumed previously (Hauser & Carvalho, 2008). However it remains that temperate marine fishes demonstrate shallow population histories, as ~~they~~ a consequence of the~~have been~~ impact~~ed by~~ of the successive glacial cycles ~~that occurred~~ during the Q~~q~~uaternary Period (Grant & Bowen, 1998; Hauser & Carvalho, 2008). Hence, their population history has ~~often~~ been ~~largely~~ affected by drift, non-equilibrium demographic patterns, and vicariant events that also promoted the creation of well-defined marine contact zones. The latter occurs when genetically differentiated~~lly~~ populations co~~a~~me into secondary contact. Moreover, patterns of population structure are complicated by variable survival rates of larval~~e~~ and post-larval stages, and hence large fluctuations in cohort size (Cushing, 1990; Hjort, 1914). This sweepstake recruitment *sensu* ~~(~~Hedgecock (~~,~~ 1994) may substantially influence how marine populations evolve~~d~~, the structure of their coalescent, and the way neutral and non-neutral processes are inferred (see ~~(~~Hedgecock & Pudovkin, 2011). Overall, the marine realm requir~~eest~~s more careful examination of the observed patterns of gene diversity~~, it~~ demand~~ings~~ an approach with a right balance between ~~large~~ the number of ~~neutral~~ loci and ~~large~~ sample size~~s~~. This should allow for a better estimate of the (outlier) markers involved in adaptive patterns (Nielsen *et al.*, 2012; Teacher *et al.*, 2013)~~,~~ by enhancing

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'neutral parametrization' (i.e. low probability to detect false positive outliers;) (Lotterhos & Whitlock, 2014).

Among the biologically well studied marine fish taxa features the European sea bass *Dicentrarchus labrax* L. (Moronidae, Teleostei), which inhabits the continental shelves of the Northeast Atlantic Ocean and Mediterranean Sea (Pérez-Ruzafa & Marco, 2014). Wild catches in the Atlantic Ocean and Mediterranean Sea reach about 910,000 and 5,000 tonnes respectively annually (FAO, 2014) while aquaculture production reaches 153 60,182000 tonnes (XXFA0, 2014). NoCatches are not regulated, apart from a few national plans, European management plan has been drawn yetbecause of poorincomplete data qualitycompilation, especially from recreational fishing mortality (www.ices.dkICES, 2004). Lately, biomass of wild Atlantic stocks has been declining rapidly while fishing mortality has been increasing leading to a recommendation for a 80% reduced effort by ICES (ICES, 2014) and the closure of the pelagic commercialtrawl fishery in January 2015.

(ref). There is strong clear evidence for the presence of an Atlantic and a Mediterranean lineage at both nuclear and mitochondrial DNA markers (Coscia *et al.*, 2012; Lemaire, Versini & Bonhomme, 2005; Naciri *et al.*, 1999; Quéré *et al.*, 2012; Tine *et al.*, 2014), with a contact zone at the Almeria-Oran front (AOF). Unlike evidence from a short cyt *b* fragment (Lemaire, Versini & Bonhomme, 2005) mitochondrial diversity based on a 6383 bp fragment is higher in the Atlantic Ocean compared to the Mediterranean Sea (π = 0.00878 and 0.00352 respectively;) Guinand (Rondon, 2011), pers. comm.). Divergence time between the Western Mediterranean and Atlantic lineages has been estimated at ca. 270,000 years BP with secondary contact at the beginning of the Holocene ca. 11,500 years BP (Tine *et al.*, 2014). Populations of the Mediterranean basin are further differentiated in an Eastern and a Western Mediterranean group separated along the Siculo-Tunisian Strait (Bahri-Sfar *et al.*, 2000; Cesaroni *et al.*, 1997; Quéré *et al.*, 2012). Except for a large mtDNA for which the level ofmitochondrial genetic differentiation is large at the contact zone at the AOF (F_{ST}> 0.70; (Coscia *et al.*, 2012; Lemaire, Versini & Bonhomme, 2005), documented overall genetic differentiation is low in sea bass. Genome-wide estimated nuclear differentiation was estimated to bate ~2.8% between the Atlantic and Western Mediterranean lineage (Tine *et al.*, 2014), and - on the basis of only twenty markers - estimated to be ~2% between the two Mediterranean basins by (Quéré *et al.*, 2012) (with no mtDNA differentiation within the Mediterranean basin; Rondon and Guinand et al., unpubl pers. comm.). Differentiation within each basin is fairly limited based on both mtDNA and nuclear markers. The Atlantic lineage shows no evidence for population expansion (Coscia *et al.*, 2012) and weak evidence for genetic structure. Samples collected along the Atlantic coasts north of Portugal by (Coscia & Mariani, 2011; Fritsch *et al.*, 2007; Quéré *et al.*, 2010;) and (Coscia *et al.*, 2012) did not show any structure, except for the British Isles (Child, 1992) –where (Child, 1992) already

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identifinoticed a genetic difference was identified at the *PGM* locus between juvenile populations from the Irish Sea and coastal UK. The western Mediterranean population is not genetically structured (Naciri *et al.*, 1999; Quéré *et al.*, 2012), while the Eastern Mediterranean contains more patchy populations (Bahri-Sfar *et al.*, 2000; Castilho & Ciftci, 2005; Quéré *et al.*, 2012), with very limited differentiation (<2%; (Bahri-Sfar *et al.*, 2000; Quéré *et al.*, 2012). Hence, as similar to many other marine fishes (e.g. (Milano *et al.*, (2014),; Ruzzante *et al.*, (2006),; Teacher *et al.*, (2013), sea bass has a shallow nuclear genetic structure that can be largely explained by neutral demographic processes (see Patarnello, Volckaert & Castilho, (2007) for a review). Nevertheless, the study of genome-wide variation in sea bass has recently demonstrated the prexistence of so-called genomic islands of differentiation (Tine *et al.*, 2014), while (Quéré *et al.*, (2012) demonstrated that markers associated with genes have larger estimates of genetic differentiation both among and within basins. If they do not contradict the very existence of neutral processesS, such findings suggest that adaptive determinisms of genetic variation are also present in sea bass, but hidden within the shallow structure observed for most marine fishes.

Hence, despite previous knowledgeowever, there areS several aspects ofn the genetic structure of European sea bass which remain unanswered. The historical Atlantic–Mediterranean pattern is unambiguously supported by several studies (e.g. (Tine *et al.*, (2014), but (i) the seemingly homogenous distribution of the Atlantic and Western Mediterranean lineages has not been analyzed basin-wide with a sufficiently large number of markers (Atlantic Ocean: max. n = 13 microsatellite loci in (Coscia & Mariani, 2011); Western Mediterranean Sea: max. n = 20 nuclear markers in (Quéré *et al.*, 2012) to conveniently partition stochastic and adaptive processes within each basin. In contrast, (ii) the observed population structure within the Eastern Mediterranean could have been overestimated because of too few markers. (iii) Some basins remain underexplored, such as the Adriatic Sea that has already been foundis known to be differentiated from the Mediterranean in several fish species (Solea solea (Garoia *et al.*, 2007;), Pomatoschistus marmoratus (Mejri *et al.*, 2011;) and Atherina boyeri (Milana *et al.*, 2012). (iv) Finally, the information content of the nuclear microsatellite and SNP markers to detect patterns has not been fully explored (but see (Quéré *et al.*, (2012) who used gene-associated markers located within 5'-flanking regions and introns of some annotated genes). Our revisit of the genetic structure of European sea bass is based on so far thea geographically most extensive sampling effort and takes advantage of a combination of nuclear putatively neutral genetic markers (existing microsatellites and newly developed SNPs) and markers putatively influenced by selection (newly developed SNPs).

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MATERIALS AND METHODS

Localities and DNA extraction

The spatial sampling design covered almost the full range of European sea bass (Figure 1, Table 1). Samples from Aveiro (P), Bardawil (Eg), Fiumicino (I), Marsala (I), Muravera (I), Rabat (Mo), Tanger - Ksar es-Seghir (Mo), Thessaloniki (Gr) and Valencia (Es) have been included in one or several previous studies: ~~(~~Allegrucci, Caccone & Sbordoni~~,~~ (1999)~~;~~ Allegrucci, Fortunato & Sbordoni~~,~~ (1997)~~;~~ Bahri-Sfar *et al.*~~,~~ (2000)~~;~~ Bonhomme *et al.*~~,~~ (2002)~~;~~ Caccone *et al.*~~,~~ (1997)~~;~~ García de León, Chikhi & Bonhomme~~,~~ (1997)~~;~~ Lemaire *et al.*~~,~~ (2000)~~;~~ Lemaire, Versini & Bonhomme~~,~~ (2005)~~;~~ Naciri *et al.*~~,~~ (1999)~~;~~ Quéré *et al.*~~,~~ (2012). Samples were collected offshore except for the lagoon~~al~~ samples from Aveiro, Bardawil, Muravera and Venice. Our sampling strategy did not explicitly aim at a temporal analysis of population structure. A small sample of either pectoral fin or muscle tissue, or scales were stored in 80% ethanol and kept at room temperature until DNA extraction. Total genomic DNA was isolated either using the Invisorb DNA Universal Clinical HTS 96 kit or the NucleoSpin Tissue Extraction kit (Machery-Nagel GmBH). DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific~~;);~~ ~~only samples with low a high DNA concentration were not genotyped~~ ~~(~~Table 1).

Genotyping of microsatellite markers

Fourteen mapped microsatellite loci were amplified and analysed: *DLA0008*, *DLA0119*, *DLA0016*, *DLA0020*, *DLA0105*, *DLA0228E*, *DLA0244*, *DLA0237*, *DLA0248*, *DLA0146*, *DLA0142*, *DLA0110*, *DLA0145* and *DLA0140* (Chistiakov *et al.*, 2005) (Table S1). ~~They~~All of them were selected based on their position on the linkage map in order to provide maximal coverage and level of polymorphism. Amplification of the loci was performed in a 20-µl polymerase chain reaction (PCR) cocktail containing *Taq* buffer 1x (Promega, 50 mm KCl, 10 mm Tris-HCl pH 9 at 25 °C, 0.1% TritonX-100), 1 mM $MgCl_2$, 150 nM of each primer, 70 µM dNTPs, 0.8 U of *Taq* and 50 ng of genomic DNA. Samples were amplified on a DNA Thermal Cycler (One-Advanced Euroclone) with the following thermal profile: (i~~a~~) Predenaturation at 94 °C for 2 min; (ii~~b~~) 30 cycles of denaturation at 94 °C for 45 s, annealing at 48 °C for 45 s, extension at 72 °C for 45 s each; (iii~~c~~) additional extension at 72 °C for 10 min. The forward primers were labelled with different fluorescent dyes allowing fragment detection on an ABI PRISM 3100 or 3700 automated sequencer (with size standard ROX-400). They were combined in two multiplex assays and the fragment analysis was run at BMR Genomics (www.bmr-

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genomics.it). Allele scoring was performed with the software GENOTYPER v~~.~~3.7 (Applied Biosystems). In order to minimize scoring errors two operators independently read and edited the program output. Only consensus genotypes (in total 98%) were retained.

Genotyping SNP markers

A total of 51 SNPs from a large data set of sequenced and *in silico* detected unique SNP candidates (Arias *et al.*, 2012; Chistiakov *et al.*, 2008; Kuhl *et al.*, 2010); L. Bargelloni, pers. comm.) wa~~s~~ere successfully genotyped with the MassARRAY system (Sequenom, San Diego, USA). PCR-primers and extension-primers were designed and optimi~~z~~sed at the Genetic Service Facility of the Flanders Institute of Biotechnology (Antwerp, Belgium). Two SNPs gave no reaction in more than 25% of the individuals and were discarded from the analysis. Similarly, 26 individuals for which more than 25% of the SNPs gave no reaction were discarded~~; they~~ and ~~were~~ excluded from Table 1.

Out of the 49 successfully genotyped SNPs, 37 SNPs were developed from Expressed Sequence Tags (ESTs) (Table S2). Ten of them were developed by resequencing several genes in ten individuals of Mediterranean origin (Chistiakov *et al.*, 2008~~)~~; D. Chistiakov, pers. com.). Twenty seven were developed by resequencing several ESTs in four individuals ~~caught~~ from~~in~~ the Mediterranean Sea and four individuals ~~caught in~~from the Atlantic Ocean (Arias *et al.*, 2012). However 12 of these SNPs were first detected in Atlantic ESTs before being validated on Mediterranean and Atlantic individuals and 15 were discovered while resequencing the ESTs on Mediterranean and Atlantic individuals. Finally, 12 SNPs were developed from BAC end sequences and validated using Mediterranean individuals (L. Bargelloni, pers. comm.). All sequences were annotated using BLASTN and BLASTX against the GENBANK database (Table S2). In order to infer distribution across the sea bass genome, sequences were mapped to the genome of European sea bass (Kuhl *et al.*, 2010; Tine *et al.*, 2014).

Genetic variation

Data quality of microsatellites was checked for null alleles, allele dropout and stuttering by using the software MICRO-CHECKER v~~.~~2.2.3 (van Oosterhout *et al.*, 2004). Data quality of SNPs was assessed by calculating the frequency at which the less common allele occurs in a given population (Minor Allele Frequency - MAF) ~~(Helyar *et al.*, 2011)~~ and by checking Mendelian inheritance of those SNPs that were polymorphic on the Venezia-Fbis family, which has been used for mapping purposes (Chistiakov *et al.*, 2008). Loci with null alleles and non-Mendelian inheritance were discarded.

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Genetic diversity was estimated for each population by calculating the mean number of alleles (A), observed and unbiased expected heterozygosities. Departure from Hardy-Weinberg expectations was assessed using Wright's inbreeding coefficient F_{IS} for each population and lineage. The significance of F_{IS} values was calculated by permutation of alleles and corrected using the sequential Bonferroni method. Significant differences between basins were tested with a non-parametric analysis of variance Kruskal-Wallis test. Linkage disequilibrium was inferred for each pair of loci within each of the 22 samples and for each pair of loci within the Atlantic and Mediterranean lineages.

Visualization of allele and genotype frequencies is done with web-based interactive geo-visualization software available at https://fishreg.jrc.ec.europa.eu/map/genetics_geobrowser (see Fig. 1). ~~One~~An interesting feature is that allele frequencies are plotted quantitatively. Various population genetic maps are available online for visualization and environmental data can be added as additional layers on the map.

Genetic structure

Genetic differentiation ~~based on all loci~~ was assessed with three approaches based on all loci in order to understand overall structure before screening for outlier markers. (i) Divergence among populations was measured using Wright's pair-wise F_{ST} values for each marker type and for samples including at least 15 individuals (but see (Kalinowski, 2005) (Willing, Dreyer & van Oosterhout, 2012). The significance of F_{ST} values was calculated by permutation of individuals and corrected using the sequential Bonferroni method. All calculations were performed using the GENETIX software v. 4.05 (Belkhir *et al.*, 1999). (ii) Individual genotypes of each marker type and the combined microsatellite and SNP markers were clustered through discriminant analysis of principal components (DAPC) (Jombart, Devillard & Balloux, 2010) as implemented in R (R Development Core Team 2014). Data was first transformed using Principal Component Analysis (PCA). After retaining an appropriate number of PCs, the k-means algorithm was run and the Bayesian Information Criterion (BIC) was used to select the most suitable *K* number of genetic clusters. As the BIC criterion is known to overestimate the number of clusters, several lower K values were tested and the value fitting the data the best was retained. Assignment of individuals to the *K* clusters and DAPC were then performed. Analyses were conducted using the ADEGENET package (Jombart & Ahmed, 2011) for the R software (http://www.r-project.org). (iii) Population structure was inferred by clustering the genotypes for all markers through running the software STRUCTURE v. 2.3.4 (Pritchard, Stephens &

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Donnelly, 2000). Unlike DAPC which maximizes genetic separation among groups and minimizes variation within groups, it groups individuals in clusters based on the minimizing of Hardy-Weinberg and linkage disequilibria. This was done for a number of populations (*K*) ranging from one to 10, using the four models available (no admixture and allele frequencies correlated, no admixture and allele frequencies not correlated, admixture and allele frequencies correlated, admixture and allele frequencies not correlated). A burn in length of 10^3 iterations and subsequently 10^4 additional Monte Carlo Markov chain (MCMC) iterations were performed. Each assessment of *K* was repeated five times to check the repeatability of the results. The most likely K, selected according to Evanno, Regnaut & Goudet (~~Evanno, Regnaut & Goudet,~~ 2005), was then used to assign each individual to its population.

Detection of outliers

~~As a standard approach, we made use of global outlier tests using all markers and all samples by basin (Atlantic and Mediterranean) to identify outlier loci representative of the whole data set. This is justified as the historic divide between the ecosystems of both basins (see introduction) interferes with the detection of contemporary selection signatures. Because a more specific aim of our study was to assess the relative importance of geographic factors in genetic divergence, we attempted to identify local selection by investigating the patterns of selection among samples within basins.~~

The approach used to detect loci influenced by directional selection is based on the expectation that they exhibit lower intrapopulation variability and larger interpopulation differentiation than neutral loci (Shikano, Ramadevi & Merilä, 2010). We investigated signatures of directional selection based on three conceptually different approaches, each set in the context of an island model, to reduce the number of false positives.

(i) To detect increased population differentiation, we adopted the hierarchical Bayesian method of F_{ST} (Foll & Gaggiotti, 2008). It estimates population-specific F_{ST} coefficients accounting for different intensities of genetic drift in the various populations. We used BAYESCAN v~~.0~~2.1. (http://cmpg.unibe.ch/software/bayescan) to perform the analyses. A total of 10 pilot runs of 5.10^3 iterations w~~a~~s~~ere~~ performed after a burn in of 50.10^3. Loci with a log10 of Bayes Factor between 1.5 and 2 and larger than 2 were considered as outlier loci with a confidence interval of 95% and 99% respectively and a q-value (i.e. minimum fa~~l~~se discovery rate) of 0.05.

(ii) In a second approach, we screened for reductions in heterozygosity between populations using the lnRH test. Indeed a reduction in heterozygosity could be caused by the occurrence of a selective sweep. LnRH tests were performed pair-wise on the microsatellite genotypes according to ~~(~~Kauer, Dieringer & Schlötterer~~,~~ (2003). LnRH estimates were standardized with a mean of 0 and a standard

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deviation of 1; 95 and 99 % of all loci are expected to have values ranging from -1.96 to 1.96 and -2.18 to 2.18 respectively. Loci with ~~lnRH~~ values outside these boundaries were considered significant. Loci were considered as outliers if they were significant in at least two pair-wise comparisons. With a false positive rate of 0.05, 25, 46, 10 and 10 false positives are expected in Atlantic, Mediterranean, Western Mediterranean and Eastern Mediterranean basins respectively.

(iii) To evaluate F_{ST} and heterozygosity at the same time, we employed a coalescent approach developed by ~~(~~Beaumont & Nichols~~,~~ (1996) as implemented in Lositan (Antao *et al.*, 2008). The parameters of LOSITAN were as follows: the confidence interval was set to 9~~5~~95 % and 99.5% with a false discovery rate set to 0.1 and 0.05 respectively, the number of permutations to 2.10^4 and the population size to 50. The infinite allele model was used for the SNP markers while the stepwise mutation model was used for microsatellite markers. In all cases, the 'neutral' mean F_{ST} was used.

All three approaches have their ~~(dis)~~advantages and sensitivities in detecting false negatives and positives, although BAYESCAN produces a low rate of false positives under a range of demographic scenarios (De Mita *et al.*, 2013; Narum & Hess, 2011). As the detection of outliers through the independent application of multiple methods increases the certainty that these are truly non-neutral, we used the information on outliers from the tests as such (without Bonferroni correction) to minimize the number of false positives. Loci were considered under directional selection when two or three tests were significant for directional selection~~, and false positive when just only one test turned out to be underidentified directional selection~~. Although the concept of balancing selection is well established, there are still methodological limitations for its identification in hitchhiking mapping (Hansen, Meier & Mensberg, 2010). Therefore we discuss only loci under directional or positive selection.

Patterns of geographical and genetic distance

A matrix of Euclidian distances (in m) was prepared by measuring the shortest distance between two adjacent sampling points over sea within a single basin (Atlantic Ocean and Mediterranean Sea). Geographic and genetic distances were compared through a partial Mantel test by opposing the geographic distance matrix to the matrix of genetic distances (F_{ST} [F_{ST}/(1-F_{ST})]; (Reynolds, Weir & Cockerham, 1983) using the Mantel coefficient Z (Rousset, 1997) in the software package IBD (Bohonak, 2002). We tested isolation by geographical distance (IBD) within the Mediterranean and Atlantic basin separately because each represents an Evolutionary Significant Unit (Lemaire, Versini & Bonhomme, 2005). Following Hemmer-Hansen *et al.* (2007) we controlled geographical distance for latitude and longitude to examine if geographical distance had the same effect in each of these dimensions. In that case population structure is best explained by a pattern of isolation by

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geographical distance. If not, it points to a minor role for geographical distance *per se*. Low sample sizes (n < 15) relative to the high allelic diversity of the microsatellite loci were excluded; they may lead to low signal to noise ratios when calculating pair-wise F_{ST} values (Kalinowski, 2005). The significance of the Mantel test was assessed by 10^6 permutations of the population in the genetic distance matrix.

~~Visualization of allele and genotype frequencies is done with web-based interactive geo-visualization software available at https://fishreg.jrc.ec.europa.eu/map/genetics_geobrowser (see Fig. 1). One feature is that allele frequencies are plotted quantitatively. Various population genetic maps are available online for visualization and environmental data can be added as additional layers on the map.~~

RESULTS ~~(1822 WORDS -> 14456814 WORDS)~~

Genetic variation of the microsatellite and SNP loci

A total of 536 individuals from 21 sites were genotyped at 14 mapped microsatellite loci; genotyping of the samples from Thessaloniki failed (Table ~~S~~1). Several sample numbers from the ~~Eastern~~ Mediterranean Sea were low, but all samples (in the case of DAPC and Structure analysis) and some samples (in the case of F_{ST} analysis; n > 15) were retained because of the valuable information. Seven out of 25 linkage groups (LG) are covered, with 3 loci mapping to LG 10 and 15. The average number of alleles per locus varied from 6.00 (Crete - GCRE) to 16.29 (Zeebrugge - BEL) and differed significantly between basins (p = 0.028). Variability at microsatellites seems higher in the Mediterranean sea than in the Atlantic Ocean. Microsatellite loci DLA105 and DLA248 showed distinct gradients in allele frequency between the Atlantic Ocean and Mediterranean Sea. Observed heterozygosity values ranged from 0.688 (Muravera - MUR) to 0.822 (Saint-Malo) among loci and did not differ between basins (p = 0.14). One sample (Aveiro - POR) appeared to deviate from Hardy-Weinberg equilibrium after Bonferroni correction (Table S3). Both the Atlantic Ocean and Western Mediterranean lineages showed a significant heterozygote deficiency over the 14 loci after Bonferroni correction. There was no linkage disequilibrium between all 14 microsatellite loci, which is congruent with their genomic position (Table S1).

~~**Genetic variation of the SNP loci**~~

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A total of 644 individuals from 22 sites were genotyped at 49 SNP loci. All but four LGs (LG 3, 10, 19 and 21) were represented by one or more SNP(s); LG 16 and LG 17 had 4 SNPs, LG 20 had 5 SNPs. The MAF ~~value~~ ranged from 0.002 for locus *Dl_6k11* to 0.487 for locus *S159*. It was lower than 5% at six loci (*Dl_6k11*, *S9*, *NS35*, *S186*, *S106* and *S109*; Table S2). Observed heterozygosity values ranged between 0.205 (Wexford - IRW) and 0.304 (Muravera - MUR), and differed significantly between basins (p = 0.006; Table S4). Out of 49 successfully genotyped SNPs, 15 were monomorphic in the mapping panel and four SNPs were homozygous in the mapping parents. ~~Two (*S110* and *S170*) out of 30 SNPs did not follow Mendelian inheritance. In addition, half of the Venezia-Fbis family could not be genotyped at locus *S218*, probably because of the presence of a null allele. Hence, L~~loci *S110*, *S170* and *S218* were discarded from further analyses because of non-Mendelian inheritance or failed genotyping, leaving the number of utilized~~seful~~ SNPs to 46 loci. None of the samples, except the Eastern Mediterranean ones, appeared to deviate from Hardy-Weinberg equilibrium after Bonferroni correction. Linkage disequilibrium permutation tests revealed that the 46 SNPs were independent, which is congruent with the genomic position of the SNPs (Table S2). At the lineage level, 24, 7 and 19 cases of linkage disequilibrium involving 24, 11 and 22 loci were significant after Bonferroni correction in the Atlantic Ocean, Western Mediterranean and Eastern Mediterranean respectively.

Genetic structure ~~**based on the microsatellite loci**~~

Overall genetic differentiation (F_{ST}) between Mediterranean and Atlantic samples was five times low~~rather high~~er based on microsatellites (0.057; p<0.001) (Table S5) than on SNP markers (0.295; p< 0.001) (Table S7) ~~and significant (0.057; p<0.001)~~. Multi-locus p; ~~p~~air-wise F_{ST} among Atlantic samples varied from 0.00~~4~~ to 0.01~~8~~8 (microsatellites) and 0.000 to 0.027 (SNPs)~~and was in all cases significant~~; ~~–~~the samples from Rabat (MRBT) and Ksar es-Seghir (MKS1) showed above average values. ~~The D~~differentiation between the Eastern and Western Mediterranean Sea was ~~also significant although~~ small (microsatellites: 0.005; p<0.001; SNPs: ~~xxx~~0.024; p<0.001) ~~although small~~. ~~Within the Atlantic basin pair-wise F_{ST} values were lower than 0.006 and notn significant after sequential Bonferroni correction, except for the samples from Rabat (MRBT) and Ksar es-Seghir (MKS1; Table S5).~~ Within the Mediterranean basin pair-wise F_{ST} values were more variable than in the Atlantic Ocean (microsatellites: 0.001<F_{ST}<0.030; SNPs: 0.000<F_{ST}<0.094), especially samples in the eastern basin vary. The presence of population structure was confirmed with assignment analysis through both DAPC and STRUCTURE. DAPC identified membership of the microsatellite genotypes to three clusters, one involving Atlantic samples and two involving Mediterranean samples (Figure 2a,b). Clustering of the SNP genotypes led to four clusters, with one cluster in the Atlantic Ocean and

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three weakly separated clusters in the Mediterranean Sea (Figure 2c, d). Almost all Atlantic individuals were assigned to the Atlantic group while almost all Mediterranean individuals ~~assigned~~ to the Mediterranean group. Individuals from the Strait of Gibraltar (Ksar es-Seghir – MKS1) clustered with the Atlantic samples. No substructure was found in the Atlantic Ocean except that three samples (Portugal - PFA, Rabat - MRBT, Ksar es-Seghir – MKS1) were influenced by Mediterranean genotypes. ~~but~~ Mediterranean samples split in two groups~~–~~: Eastern and Western Mediterranean basin (Figure 2a, b) or an additional Adriatic group in case of the SNP genotypes (Figure 2c, d). Results from the STRUCTURE analysis are comparable to the DAPC analysis with k = 2~~3~~ providing the most probable groupings including one Atlantic and ~~two~~ one Mediterranean group~~s~~ (Figure S~~S~~1, S2). ~~The analysis by basin did not show any substructure neither in the Atlantic Ocean nor in the Mediterranean Sea.~~Two groups were detected in the Atlantic basin based on the SNP markers with the samples of Faro (PFA) and Morocco (MRBT and MKS1) constituting a separate cluster (Figure 1, S3). Although the number of clusters of the SNP genotypes was evaluated to be two in the Mediterranean basin, ~~no clear~~a weak substructure of three groups was observed (Figure 1, S4b).

We restricted the combined analysis of the microsatellite and SNP genotypes to DAPC, due to different assumptions underlying the analysis of the two marker types in STRUCTURE. Overall, population structure was confirmed. The scatter plot of the first two components of the DAPC fitted a hierarchical island model. The number of genetic clusters was parsimoniously estimated to be four, similar to the analysis based on SNP genotypes and one more than the microsatellite genotypes. One cluster grouped all Atlantic samples while the three others clustered all Mediterranean samples. Samples from each basin formed distinct populations, and the Mediterranean samples split in a western, Adriatic and eastern group (Figure 2e, f). Samples from Morocco (MRBT and MKS1) clustered with the Atlantic lineage while the Murcia (EMUR) sample clustered with the Mediterranean lineage. Genotypes of a few individuals from Valencia (EGLV) and Sète (FSET) clustered with the Adriatic group.

Mantel tests indicated that Atlantic samples were isolated by distance (microsatellites: Z = 196,2605~~9~~; r = 0.379; p = 0.04; SNPs: Z = 653598; r = 0.683; p = 0.0001). The correlation of genetic distances to geographic distances remained significant when controlling for latitude (microsatellites: r = 0.371; p = 0.04) and longitude (microsatellites: r = 0.388; p = 0.03; SNPs : r = 0.799; p = 0.003). ~~Latitude was significantly correlated to genetic distance whether controlling for geographic distance (r = 0.555; p = 0.04) or not (Z = 1.7; r = 0.559; p = 0.002). Longitude was not significantly correlated to genetic distance (Z = 0.5; r = -0.119; p = 0.41).~~ There is a measurable effect of the southeast Atlantic

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samples of Rabat (MRBT) and Ksar es-Seghir (MKS1) as none of the correlations remained significant after their removal (Table S6).

~~A correlation between~~G~~ g~~enetic and geographic distances ~~was found~~o ~~f~~~~or~~ the Mediterranean samples (microsatellites: Z = 1,710,752; *r* = 0.509; *p* = 0.04; SNP: Z = 4,309,638; r = 0.643; *p* = 0.0006) and~~,~~ Eastern Mediterranean samples (microsatellites: Z = 283369; *r* = 0.899; *p* = 0.04; SNP: Z = 830887; r = 0.815; *p* = 0.02) were correlated but not ~~for~~ the Western Mediterranean samples (microsatellites : Z = 165783; *r* = 0.167; *p* = 0.21; SNP: Z = 65774; r = -0.221; *p* = 0.01). None of the correlations were significant when controlling for either latitude or longitude in case of the microsatellites~~.~~ ~~Genetic distances were significantly correlated with longitude in Mediterranean (Z = 13.5; r = 0.646; p = 0.03) and Eastern Mediterranean (Z = 2.2; r = 0.892; p = 0.04) samples but only the correlation in Mediterranean samples remained significant after controlling for geographic distances (r = 0.463; p = 0.04). Latitude was significantly correlated to genetic distances for Eastern Mediterranean samples (Z = 1.5; r = 0.839; p = 0.04) but the correlation was no longer significant when controlling for geographic distances~~ (Table S6).

~~**Genetic structure based on the SNP loci**~~

~~All 46 SNPs were used to evaluate Atlantic and Mediterranean population structure (Table S7). Overall genetic differentiation (F_{st}) between Mediterranean and Atlantic samples was high and significant (0.295; *p*< 0.001) (Table S7). The differentiation between the Eastern and Western Mediterranean Sea was also significant but ten times smaller (0.024; *p*< 0.001). Multi-locus pair-wise F_{st} ranged from 0 to 0.027 between Atlantic samples, from 0 to 0.094 between Mediterranean samples and from 0.170 to 0.444 between pairs of Atlantic-Mediterranean samples. Values were considerably higher than in the analysis with the microsatellite loci. Within the Atlantic basin F_{st} values were smaller than 0.007, except for the samples from Rabat (MRBT) and Ksar es-Seghir (MKS1). Within the Mediterranean basin F_{st} values were more variable (0.000 < F_{st}< 0.072). The presence of population structure was confirmed with assignment analysis through both DAPC and STRUCTURE. The number of genetic clusters making a clear separation as detected by ADEGENET was four, with one cluster in the Atlantic Ocean and three weakly separated clusters in the Mediterranean Sea (Figure 2c, d). Differentiation between the Mediterranean Sea and Atlantic Ocean was sharper than with the microsatellite loci. No substructure was observed in the Atlantic samples except that three samples (Portugal – PFA, Rabat – MRBT, Ksar es Seghir – MKS1) were influenced by a slight gene flow from the Mediterranean Sea. Three groups were identified in the Mediterranean Sea (Western Mediterranean, Adriatic and Eastern Mediterranean; Figure 1). The sharper~~

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Comment [Erika Sou6]: Should we add here that the structure analysis performed after removal of SL-UTR SNP gave the same results?

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segregation of the Mediterranean SNP genotypes in three groups contrasts with the microsatellite genotypes discerning two groups. Results match with the STRUCTURE analysis which also distinguished clearly between Atlantic and Mediterranean samples (*K*=2 when all samples were considered; Figure S2). Two groups were detected in the Atlantic basin, the samples of Faro (PFA) and Morocco (MRBT and MKS1) constituting a separate cluster (Figure 1). Although the number of clusters was evaluated to be two in the Mediterranean basin, no clear substructure was observed.and differentiated between three groups in the Mediterranean Sea (Figure S2). The sharper segregation of the Mediterranean SNP genotypes in three groups contrasts with the microsatellite genotypes discerning two groups.

Atlantic samples were isolated by distance (Z = 653598; *r* = 0.683; *p* = 0.0001); the correlation remained significant after controlling for longitude (*r* = 0.799; *p* = 0.003) but not for latitude. Latitude was significantly correlated to genetic distance (Z = 4.9; *r* = 0.736; *p* = 0.001) and the correlation remained significant when controlling for geographic distances (*r* = 0.405; *p* = 0.018). Nonet any of these correlations remained significant after the removal of the Rabat (MRBT) and Ksar es-Seghir (MKS1) samples from the data set (Z = 49669; r = 0.277; *p* = 0.17) (Table S6).

Mediterranean samples were also isolated by distance (Z = 4309637; *r* = 0.643; *p* = 0.0006). Similar to microsatellites, IBD was significant in the eastern basin (Z = 830887; r = 0.815; *p* = 0.02) but not in the western basin (Z = 65774; *r* = -0.221; *p* = 0.01). Correlations remained significant when controlling for latitude in Mediterranean samples (*r* = 0.536; *p* = 0.001) and Eastern Mediterranean samples (*r* = 0.713; p = 0.03) and when controlling for longitude in Mediterranean samples (*r* = 0.541; *p* = 0.01). Both latitude and longitude were significantly correlated to genetic distance in the Mediterranean samples (latitude: Z = 12.1; r = 0.485; *p* = 0.01; longitude: Z = 29.5; *r* = 0.432; *p* = 0.03) and Eastern Mediterranean samples (latitude: Z = 3.8; r = 0.603; *p* = 0.05; longitude: Z = 5.8; *r* = 0.751; *p* =0.01) but no correlation remained significant when controlling for geographic distance.

Genetic structure based on the microsatellite and SNP loci combined

We restricted the combined analysis of the microsatellite and SNP genotypes to DAPC, due to different because of assumptions runderlyling the multi-allelic microsatellite and bi-allelic SNPanalysis of the two marker types in STRUCTURE genotypes. Overall, the presence of population structure was confirmed. The scatter plot of the first two components of the DAPC fitted a hierarchical island model. The number of genetic clusters was parsimoniously estimated to be four, similar to the analysis based on SNP genotypes and one more than the microsatellite genotypes. One

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~~cluster grouped all Atlantic samples while the three others clustered all Mediterranean samples. Samples from each basin formed distinct populations, and the Mediterranean samples split in a western, Adriatic and eastern group (Figure 2e, f). Samples from Morocco (MRBT and MKS1) clustered with the Atlantic lineage while the Murcia (EMUR) sample clustered with the Mediterranean lineage. A few individuals from Valencia (EGLV) and Sète (FSET) tended had genotypes to showwhich clustered with the Adriatic group genotypes.~~

Detection of outlier loci

~~For all lnRH tests~~T~~, t~~he number of comparisons in which loci were detected under selection was lower than the number of false positives expected with a false positive rate of 0.05 in all lnRH tests. Global outlier tests by lineage showed weak signatures of directional selection at 2/0/~~9~~0 microsatellite and 6~~8~~/0 SNP loci with lnRH/LOSITAN/BAYESCAN and LOSITAN/BAYESCAN respectively in the Atlantic Ocean (Table 2). The signal w~~is amounted gaveto~~ as stronger in the Mediterranean lineage ~~signal of~~at 6/3/~~9~~0 and 3~~5~~/1 with lnRH/LOSITAN/BAYESCAN and LOSITAN/BAYESCAN respectively ~~in the Mediterranean lineage~~. Because of ~~published evidence for~~ the sharp distinction between the two Mediterranean basins, the Mediterranean samples were analysed separately in a Western and an Eastern Mediterranean group. Here the significant outlier tests were 2/0~~2~~/4~~0~~ and 2~~4~~/0 for the Western Mediterranean and 3/1/~~0~~1 and 1~~4~~/1 for the Eastern Mediterranean respectively. ~~Most loci were significant for just one outlier test, which may point to false positive results. Among the microsatellite loci, twosix loci (*DLA0008* – LG 24, *DLA0119* – LG 14, *DLA0142* – LG 15, *DLA0145* – LG 17, *DLA0146* – LG 10 and *DLA0248* – LG 15) were identified as strong candidates for directional selection in two (but not the BAYESCAN analysis) of the three tests in the Mediterranean population. However~~S ~~s~~ince the number of comparisons in which outlier loci were detected ~~under selection~~ was smaller than the number of expected false positives, those loci are probably not under directional selection. Among the SNP loci, just one locus (*SL-UTR1*; somatolactin; LG 13) was identified as a strong candidate in the Mediterranean lineage and in the Eastern Mediterranean group. Unlike elsewhere frequencies of allele *SL-UTR1-1* were higher than 0.3 in the Adriatic Sea and Northern Mediterranean Sea. As aquaculture escapees have been identified in the Bardawil ~~sample has been shown~~ (Bahri-Sfar et al., 2005) and are suspected in the Messolonghi sample ~~is suspected~~ (Dimitriou et al., 2007) ~~to involve escapees~~, the analysis was repeated without these samples. The LOSITAN analysis still recognized locus *SL-UTR1* as outlier (data not shown). Its allele frequencies are consistently grouped in an Eastern ~~Mediterranean~~ and ~~a~~ Western Mediterranean area. An analysis of genetic structure without the *SL-UTR1* locus resulted in the same results as mentioned above.

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DISCUSSION ~~(3060 WORDS → 2795713 WORDS)~~

In European sea bass, a set of 14 microsatellite and 46 SNP markers yielded well known and new patterns of intrapopulation genetic differentiation ~~comparable to, although with more evidence for structure than, 14 microsatellite markers~~. As expected, ~~our results confirm the presence of~~ there is a clear difference between the~~an~~ Atlantic and Mediterranean lineage. New knowledge is that the Atlantic lineage ~~inhabiting the Atlantic Ocean~~ has a weak structure; it ~~and~~ is ~~influenced~~ introgressed in the southeastern range of the Atlantic Ocean by the Mediterranean lineage. Sea bass inhabiting the Mediterranean basin ~~shows evidence of isolation by distance~~is structured in three groups~~; the western Mediterranean, eastern Mediterranean and Adriatic Sea are slightly differentiated~~. The western Mediterranean population ~~is~~seem~~s~~ homogenously structured, while the eastern Mediterranean shows evidence of isolation by distance. ~~Putative directional~~Signatures of selection at two microsatellite loci and one SNP locus associated with the 3'UTR of the somatolactin gene characterize~~s~~ the Eastern Mediterranean group.

The power of SNP and microsatellite markers to detect historical and contemporary spatial patterns

Our study combines a set of 46 mapped and annotated SNPs with an established resource of 14 mapped anonymous microsatellite markers (Chistiakov *et al.*, 2005; Chistiakov *et al.*, 2008). SNPs and microsatellite markers are firmly established in population genetics, each ~~with~~ having distinct and complementary characteristics~~. Microsatellites outperform SNPs because the information content of SNPs is lower due to biallelic variation at each nucleotide (Hess, Matala & Narum, 2011; Liu et al., 2005). However, the technically more reliable scoring and screening of SNPs, the simple infinite sites model of mutation, the lack of significant homoplasy and the hundreds of thousands of loci available makes them increasingly accepted in non-model organisms~~ (Hagen *et al.*, 2013; Helyar *et al.*, 2011; Morin, Luikart & Wayne, 2004). W~~Hence w~~ith SNPs outperforming microsatellites at a finer scale in non-model organisms, greater power has been achieved to discriminate populations with SNP and microsatellite markers combined (Hess, Matala & Narum, 2011) or with genome scans based on thousands of SNP markers (Corander *et al.*, 2013; Roesti *et al.*, 2014). Overall, our 14 microsatellites and 46 SNPs harbor complementary information on genetic diversity and structure, with SNP observed heterozygosities being on average three times lower, estimates of F_{IS} on average

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comparable although not always congruent, and F_{ST} values on average several times higher between basins. Our values of genetic differentiation at the microsatellite loci (overall F_{ST} = 0.041) match published data of European sea bass (Coscia & Mariani, 2011; Fritsch *et al.*, 2007; Lemaire, Versini & Bonhomme, 2005; Quéré *et al.*, 2012). SNP loci show higher values (overall F_{ST} = 0.194)~~,~~ although three to four times lower than for mtDNA (Coscia *et al.*, 2012; Lemaire *et al.*, 2000). SNP genotypes separated the North African samples from the other Atlantic samples and distinguished better between Adriatic, Western and Eastern Mediterranean samples. The combination of microsatellites and SNP genotypes provided a similar picture as the SNP genotypes, although the pattern changed somewhat in the Mediterranean Sea.

The power of markers required to discriminate among "open" marine populations is ~~highly~~ link~~relat~~ed to their ~~nature~~ characteristics and ~~numbers~~the degree of differentiation. ~~Although some argue that a small number may suffice (Provan *et al.*, 2013),~~ Iincreasing the number of markers from tens of microsatellites to thousands of SNPs has enhanced the resolution and confidence in individual genotypes (Novembre *et al.*, 2008), a~~,~~lthough small numbers may suffice (Provan *et al.*, 2013), especially in the case of SNPs (Willing, Dreyer & van Oosterhout, 2012). ~~The papers of (Larson et al., 2014; Narum & Hess, 2011) are representative of the historical progression in genotyping tools from allozymes to SNPs used to discriminate chinook salmon Oncorhynchus tshawytscha at increasingly higher resolutions. As such it is representative for many other taxa. This study makes use of tens of gene-associated SNP markers and hence fits in a sequel of genetic studies on marine fish which can be considered transitional, but which represents a step forward in European sea bass. In principle, microsatellite and SNP markers are equally useful, but SNPs are considerably more practical. Therefore~~N~~ew genomic venues have been opened with a recent study of European sea bass based on 234,148 SNPs isolated through ~~reduced~~ restriction enzyme ~~representation~~ associated DNA (RAD) genotyping ~~(RAD sequencing) has opened new genomic venues~~ (Tine *et al.*, 2014).

Genetic patterns in the Atlantic Ocean

Detecting subtle genetic structure in outbred marine species with large effective population sizes requires careful analysis of neutral and non-neutral genetic variation in order to understand the balance between geographical fragmentation, connectivity and adaptation (André *et al.*, 2011; Hemmer-Hansen *et al.*, 2013; Pujolar *et al.*, 2014). In agreement with the literature on European sea bass (see introduction) we found evidence for a sharp separation between the Mediterranean Sea and Atlantic Ocean with microsatellite and SNP markers alike. Separation has been attributed to vicariance during the Pleistocene due to changing sea levels and hydrodynamic patterns, and shifting

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climate zones. ~~While evidence for two lineages of European sea bass is firm (Bonhomme *et al.*, 2002; Lemaire *et al.*, 2000; Naciri *et al.*, 1999; Quéré *et al.*, 2012; Tine *et al.*, 2014),~~ gGenomic analysis has revealed secondary nuclear ~~secondary~~ introgression from the Atlantic to the Mediterranean lineage (Tine et al., 2014) and cytoplasmic secondary introgression from the Mediterranean to the Atlantic lineage (Lemaire, Versini & Bonhomme, 2005) following re-established contact in the Holocene. The Almeria-Oran hydrodynamic front (AOF) functions as a barrier, separating cold and less saline Atlantic water from denser Mediterranean water masses. Many, although not all, marine taxa are affected by this strong environmental barrier (see review of ~~(~~Patarnello, Volckaert & Castilho~~,~~ 2007).

So far no distinct genetic spatial pattern of the Atlantic lineage has been detected, although ~~the~~ a large~~5000 km long~~ latitudinal range from ~~off~~ 33° N (southern Morocco~~) up~~ to 60° N~~middle~~ (Norway) ~~leaves room for spatial segregation~~was sampled. Southern populations did not seem to have retained an ancestral identity under influence of latitudinal range shifts during the Pleistocene. ~~(~~Kettle *et al.*~~,~~ (2011) identified in the southeastern ~~range of the~~ Atlantic Ocean two ~~distinct~~ refuges ~~based on several cases~~: the Azores, Canaries and NW Africa, and the Atlantic Iberian peninsula (Borrero-Perez *et al.*, 2011; Chevolot *et al.*, 2006; Roman & Palumbi, 2004; Xavier *et al.*, 2011). The weak pattern judged from F_{ST} values, tests for isolation by distance and assignment analysis do not suggest vicariance but ~~spilling over~~gene flow of nuclear genetic material from the Mediterranean Sea into the Atlantic Ocean (as observed at Rabat and Ksar es-Seghir. While ~~(~~Tine *et al.*~~,~~ (2014) document the introgression of the Atlantic nuclear genome into the Mediterranean, our evidence points ~~also~~ to introgression in the opposite direction. This is~~,~~ in agreement with previous studies~~.~~ (Coscia *et al.*, 2012; Coscia & Mariani, 2011; Lemaire, Versini & Bonhomme, 2005), ~~point to~~which identify introgression of mitochondrial genomes from the Mediterranean Sea in the Atlantic Ocean. ~~The latter authors make a strong argument for introgression of from the Mediterranean lineage based on the nuclear and mitochondrial genomes.~~ This raises an interesting question on the permeability of the ~~Almeria-Oran frontal~~AOF system whose stability is influenced by seasonal variation in coastal currents. There is no further evidence for sea bass in the literature as unfortunately the coast of North Africa remains vastly undersampled (but see ~~(~~Bonhomme *et al.*~~,~~ 2002~~;~~ Lemaire, Versini & Bonhomme~~,~~ 2005~~;~~ Naciri *et al.*~~,~~ 1999).

European sea bass has the capacity to ~~makes long~~ migrat~~e~~ions ~~of~~ up to several hundred kilometers to the spawning grounds along the Northeastern Atlantic coasts ~~up to several hundred kilometers along the Northeastern Atlantic coasts to the spawning grounds~~ (Fritsch *et al.*, 2007; Pawson *et al.*, 2008; Pickett & Pawson, 1994). The latter authors attribute the ~~paradox of a~~ lack of genetic differentiation ~~notwithstanding evidence for distinct stocks in the Bay of Biscay, English Channel and Celtic Sea~~ to low level exchanges between populations. Also ~~(~~Coscia & Mariani~~,~~ (2011), covering sea bass

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populations from the Bay of Biscay up to Norway ~~and genotyped at 13 microsatellites (of which loci *DLA0110* and *DLA0119* are shared with this study)~~, revealed a homogenous genetic structure. ~~Indeed~~However, ~~such~~ the microsatellite-based genotypes integrate historical (Holocene) gene flow and contemporaneous exchange~~s~~, and are limited in their power to separate populations. With non-neutral markers differentiation at a finer resolution can be detected. For example, ~~(~~Quéré *et al.*~~,~~ (2010) found the somatolactin (SL) gene to differentiate the Bay of Biscay from the southern North Sea while other loci associated to candidate genes did not. Although the SL locus was included in our study, albeit genotyped with a different marker, we could not confirm the latter pattern.

The conservation implications of the weakly structured and heavily exploited sea bass populations are twofold. First, although ~~as yet genetic populations are not sharply separated~~we find no genetic differentiation, ~~this does not prevent the delineation~~we favor the ~~of management~~delineation of ~~various~~ spawning stocks (Pawson, Kupschus & Pickett, 2007; Reiss *et al.*, 2009). Hence, we concur with ~~(~~Pawson *et al.*~~,~~ (2007) to assign stock units in the North Sea, the Eastern English Channel, the Western English Channel, and the combined Irish and Celtic Sea. We ~~like~~ propose ~~to add~~ three ~~more~~ additional stocks: the Bay of Biscay ~~(~~following ~~(~~Quéré *et al.*~~,~~ (2010), the coasts off Portugal to Morocco~~,~~ and the Alboran Sea (this study). We speculate that ~~it might include~~ the so far unexplored transition zone between Northwest Iberia (*Neiva et al.*, 2012) and Cape Sagres (Portugal) (Castilho & McAndrew, 1998; Martinez *et al.*, 1991) might also play a role in ~~identifying~~ stock~~s~~. delineation. As stock assessments under the guidance of ICES have only recently been introduced for the data-limited stocks of sea bass, management measures have been implemented under the precautionary rule (ICES, 2014). It is expected that with access to numerous ~~genomic~~ high-resolution markers (Tine *et al.*, 2014); project AQUATRACE – https://aquatrace.eu) the subtle genetic pattern of European sea bass ~~should~~ will become better ~~understoodresolved~~understood.

The second aspect of conservation relates to climate change and its effects on the population dynamics at the ~~edges~~ border of the distribution range. European sea bass has steadily expanded its range into northern Atlantic waters (Pawson, Kupschus & Pickett, 2007)~~.~~ ~~Distribution shifts are a common adaptive strategy~~ in response to changing local conditions (Beaugrand *et al.*, 2013; Cheung, Watson & Pauly, 2013; Davis & Shaw, 2001). There is also the putative disappearance of populations in the southern range, although without any firm evidence ~~for sea bass~~ due to ~~the~~ limited research ~~off~~ in North African waters. Here the unique southeastern population merits close attention because global change impacts dramatically populations at the southern ~~edge~~ border of their distribution range (Provan & Maggs, 2012; Xavier *et al.*, 2011). ~~Unfortunately our sampling strategy does not allow for temporal analysis of population structure.~~

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Genetic patterns in the Mediterranean Sea

Two main physical features have shaped the biogeographyta of the oligotrophic Mediterranean Sea. First, the Western and Eastern Mediterranean Sea have been influenced by different oceanographic conditions throughout the Pleistocene and Holocene era (Patarnello, Volckaert & Castilho, 2007). Geographically separate units (refuges) have led to incipient allopatric speciation as observed in the phylogeographical and phylogenetic patterns of taxa such as the fan mussel *Pinna nobilis* (Sanna *et al*., 2013), the seagrass *Posidonia oceanica* (Arnaud-Haond *et al*., 2007) and *Pomatoschistus* gobies (Larmuseau *et al*., 2010; Mejri *et al*., 2011). Second, water masses vary in salinity and temperature along a west to east gradient and have been influencing spatial divergence, for example in European hake *Merluccius merluccius* (Milano *et al*., 2014) and Atlantic Bluefin tuna *Thunnus thynnus* (Riccioni *et al*., 2013). In the case of sea bass, Pleistocene cycling doesn't seem to have had that much an influence while contemporary environmental features have impacted Mediterranean populations to some degree. They are fragmented in three slightly differentiated groups, each of them associated with a subbasin. As expected, European sea bass samples split in an eastern and western group, each linked to the Eastern and Western Mediterranean basin respectively. The Siculo-Tunisian transition represents the boundary between both, similar to several other taxa such as seagrass *Posidionia* (Serra *et al*., 2010) and the goby *Pomatoschistus tortonesei* (Mejri *et al*., 2009). The separation of sea bass fits with previous evidence from allozyme markers (Allegrucci, Fortunato & Sbordoni, 1997) and microsatellite markers (Bahri-Sfar *et al*., 2000; Quéré *et al*., 2012). Interestingly mM4itochondrial DNA polymorphisms and differentiation do not differ between basins (Guinand Rondon, 2011et al., unpubl.).

European sea bass caught in the Western Mediterranean Sea show genetic homogeneity (García de León, Chikhi & Bonhomme, 1997; Lemaire, Versini & Bonhomme, 2005; Naciri *et al*., 1999), a feature that has been attributed to hybrid swarming (Quéré *et al*., 2012). (Bierne et al., 2011) and (Tine et al., 2014) contend thatA allopatric populations of the Atlantic Ocean and Eastern Mediterranean Sea seemcame to have come into secondary contact in the Western Mediterranean Sea and introgressed asymmetrically (Bierne *et al*., 2011; Tine *et al*., 2014). The asymmetrical introgressionIt is not only obvious in the allelic profile but also inand the genomic architecture.

The second group, inhabiting in the Adriatic basin has been overlooked previously, largely because no sample from that region had been incorporated in the analyses, except for a single sample from Venice (but see Bahri-Sfar *et al*., 2000). However Tthe subtle differentiation is probably basedshows a pattern ofn isolation by distance probably in response to the local physical oceanography. The

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Pelagosa Sill and Strait of Otranto have been determining factors in isolating the biota of the Adriatic Sea by~~; currents are organized~~forming ~~in~~ a northern and central cyclonic gyre ~~(Schiavina et al., 2014)~~. For example, the distribution of the Mediterranean shore crab *Carcinus aestuarii* fits the current pattern ~~of the currents~~ and splits in three populations, each matching with a ~~cyclonic~~ gyre (Schiavina *et al.*, 2014). However, ~~the~~ European sea bass inhabiting the Adriatic Sea did not ~~seem to split~~show evidence of ~~in smaller~~ additional subdivision~~units~~. ~~Hence, the subtle fragmentation is probably of a recent origin unlike the h~~. ~~Historical patterns such as observed in the phylogeography of plankton (*Calanus helgolandicus* (Yebra *et al.*, 2011), benthos (*Orchestia montagui* (Pavesi *et al.*, 2013) and fishes (sole *Solea solea* (Garoia *et al.*, 2007)) do not seem to apply.~~

European sea bass shows more genetic structure in the Eastern Mediterranean Sea than in any other basin, although some small sample sizes could have influenced the outcome. The Messolongi fish has two genetic backgrounds, which might be attributed to escapees from local aquaculture (Dimitriou *et al.*, 2007). The above average genetic distances observed by (Bahri-Sfar *et al.*, 2000; Castilho & Ciftci, 2005; Quéré *et al.*, 2012) fit the isolation by distance pattern we observed. An alternative interpretation that local populations bear the impact of a fragmented geography at low sea level stands during the Pleistocene and hence changing currents and water masses (Rohling *et al.*, 2014) seems less likely. Unlike the mitochondrial haplotypes, only the microsatellite and SNP genotypes show some structure. Also, the low number of microsatellite markers used in earlier studies might have artificially inflated differentiation ~~in older studies~~.

~~The~~A possible role ~~of~~for a candidate gene: somatolactin

An interesting outcome is the geographical distribution of the outlier locus somatolactin (SL). It is increasingly appreciated in natural populations that specific genomic regions underlie variation in adaptive traits and hence may be used to delineate population units (Funk *et al.*, 2012)~~Hemmer-Hansen et al., 2014)~~. Especially non-neutral markers, often linked to and possibly affected by local environmental conditions, have been effective in revealing subtle patterns~~. This was even more so the case~~ in the ocean with its high potential for advection and animal dispersal (Cimmaruta *et al.*, 2005; Nielsen *et al.*, 2012; Hemmer-Hansen *et al.*, 2014). The challenge has been to link allelic variance and phenotypic change functionally, either through linkage analysis (Quantitative Trait Loci), ecological genetic approaches or combined field and lab based studies. Increasingly c~~C~~ases with good evidence of genes ~~involved~~implicated have been documented (e.g., ~~rhodopsin involved in vision Sugawara et al. 2005; Larmuseau~~ (Larmuseau *et al.*, 2009)~~*et al.*, 2009), ectodisplasin involved in xxx (Jones~~ (Jones *et al.*, 2012)~~et al. 2012), MHC involved in immunity (Eizaguirre~~ (Eizaguirre *et al.*,

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2012)et al.) and cytochrome P4501A in detoxification (Williams (Williams & Oleksiak, 2011)and Oleksiak, 2011) are increasing in umber. While so far only the *EIF3E* marker gene seems to be involvedhas been identified to play a role in local adaptation of sea bass to salinity (Guinand (Guinand *et al*., 2015)et al. 2015), the evidence of the growth hormone, prolactin and somatolactin genes of several marker genes on a regional scale (Quéré *et al*., 2010) has been criticized on the grounds of experimental bias (Guinand *et al.*, 2015). However, the somatolactin hormone identified previously, appears also in our genome scan.

The hormone somatolactin is expressed in the pituitary and involved in a broad spectrum of functions including maturation, calcium regulation, body-color regulation, lipid metabolism, cortisol secretion *in vivo*, acid-base regulation, fat metabolism and background adaptation (e.g., (Kaneko & Hirano, (1993),; Vargas-Chacoff *et al*., (2009)). Especially It plays as significant role in the osmoregulation has been acknowledged iofn sea bass (Varsamos *et al*., 2006). The architecture of the sea bass somatolactin gene (*dlSL*) includes five exons and a promoter region with a polymorphic SSR and several transcription factor binding sites (including *cis*-regulatory elements such as Pit-1a) (Quéré *et al*., 2010). SNP *SL-UTR1* genotyped in this study is located in the 3' untranslated region, 220 bp removed from the end of the coding sequence. A second SNP was found 82 bp from the first SNP. While SSR polymorphism might modulate SL expression, in addition to other elements located either upstream in of the promoter or elsewhere in (non-)coding regions might also play a role. In this study, the significant differences between the Eastern and Western Mediterranean population at the somatolactin locus originate from higher values of the A allele in the Adriatic, Ionian and Aegean Sea, which are regions of more variable salinities along the longitudinal salinity gradient. (Quéré *et al*., (2012) reported a clinal pattern of genetic differentiation possibly supporting adaptive variation at one single anonymous microsatellite locus (*DLA0068*) among the twenty-one loci of sea bass studied (but see Guinand *et al*., (2015)). Xxx In Tthe only otherre is one documented case of hake in the Mediterranean Sea suggests that; (Cimmaruta, Bondanelli & Nascetti, 2005) suggested that European hake carrying the *Gapdh*^{120} and *Gpi2*^{96} alleles might be better adapted to a higher salinity (Cimmaruta, Bondanelli & Nascetti, 2005). However, given that the long separate evolutionary histories in the Mediterranean and Atlantic basins under diverse environmental conditions sea bass have ledevolved to phenodistinct ecotypes ((Gorshkov *et al*., 2004; Mylonas *et al*., 2005; Vandeputte *et al*., 2014)ref), with a distinct the genomic architecture (Tine *et al*., 2014) of sea bass should harbor a number of distinct evolutionary signals.

In summary, nuclear markers support the division of the Mediterranean populations of European sea bass in a western, Adriatic and eastern group. Howeverwhich, this is more likely the result of spatial than of historical segregation. Two points should be mentioned in regards to the management of the

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sea bass stocks. First, the management of the commercial and recreational fishery should take into account the revealed (genetic) stock structure. While this study identified three genetically distinct stocks in the Mediterranean Sea, the eastern Mediterranean basin and the Black Sea might harbor additional diversity. ~~There is also strong evidence for within-cohort differentiation between offshore and inshore populations (Allegrucci, Fortunato & Sbordoni, 1997).~~ Accordingly, species-specific quota and ecosystem based management should account for this by implementing at least ~~four~~ three management units: Western Mediterranean Sea, Eastern Mediterranean Sea and~~,~~ Adriatic Sea ~~and Black Sea~~.

Second, massive anthropogenic impacts on the natural populations of European sea bass raise major concerns. Expanding aquaculture ~~of domesticated and selected sea bass~~ in ~~open~~ sea pens is ~~thought to be~~ associated with massive escapes (Arechavala-Lopez *et al.*, 2011), a phenomenon also observed in other marine fish (Jørstad *et al.*, 2008). Overall documentation is poor ~~There are~~regardless of~~e~~ testimonies and occasional records based on ecological (Toledo-Guedes, Sanchez-Jerez & Brito, 2014) and genetic evidence (Bahri-Sfar *et al.*, 2005; Katsares *et al.*, 2005; Triantafyllidis, 2007)~~, but overall documentation is poor~~. Concerns for genetic introgression are high becaus~~As~~e domesticated sea bass have a different phenotypic and genetic profile than their natural conspecifics. This is~~,~~ either because of the source of the stock or because of intensive selection. As a consequence~~, because~~ reared fish ~~are~~ may be sources ~~for~~ of local infections (Arechavala-Lopez *et al.*, 2013) and ~~because~~ interbreeding w~~between~~ ith wild ~~and escaped sea bass is likely~~conspecifics, especially in the vicinity of the spawning grounds~~, concerns for genetic introgression are high~~. We ~~found~~ could not test~~no evidence~~ for escapees because our analysis did not include reference samples from aquaculture and our genome sampling is too sparse to detect hybrids.

~~Another anthropogenic factor raising concern for the natural populations of sea bass and the communities they are associated with is the massive influx of Lessepsian immigrants (Galil *et al.*, 2014). Several non-indigenous species (NIS) occupy niches associated with the juvenile and adult stages of European sea bass. So far, documentation is sparse, but because of the common occurrence of sea bass and the expanding populations of NIS in coastal areas, concerns are grave.~~

In conclusion, two distinct lineages of European sea bass inhabit the Atlantic Ocean and Mediterranean Sea. Within each basin isolation by distance has further shaped the spatial structure; the pattern is ~~faint~~ weak in the Atlantic Ocean and shows evidence for three~~sharper~~ divisions in the Mediterranean Sea, most~~. The genetic structure of the Mediterranean Sea is~~ likely the result of geographical isolation. Further genomic studies might resolve this standing question, together with ~~additional~~ other open questions related to the nature of introgression in the Atlantic-Mediterranean

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contact zone, adaptation to longitudinal and latitudinal gradients, the ~~differentiation~~ relationship between~~in~~ lagoon~~al~~ and offshore populations, and the impact of fishing pressure.

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CONFLICT OF INTEREST

None of the authors declares a conflict of interest.

DATA ARCHIVING

SNP and microsatellites genotypes have been deposited in the DRYAD databank: doi:xxx.

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Figure 1 Location of samples and sample-specific genetic differentiation by basin of *Dicentrarchus labrax* based on assignment analysis in STRUCTURE with SNP markers. (a) Atlantic Ocean; (b) Mediterranean Sea. The colors used to represent the frequencies are derived from the CMYK color model. One of each of the colors cyan, magenta, yellow and black are assigned to each of the clusters represented. Colors are mixed according to the proportions of the representative clusters at each geographic location. The graph in the bottom left hand corner of the display shows the precise proportion of each cluster at the represented location. For site locations see Table 1. For full color representation consult the e-paper.

Figure 2 DAPC-based clustering of microsatellite genotypes (a and b), SNP genotypes (c and d) and the combined microsatellite and SNP genotypes (e and f) of European sea bass. Panels a, c and e show the DAPC plot of the clusters and panels b, d and f show the results for membership probability per individual organized by sampling site. See Table 1 for codes. For full color representation consult the e-paper.

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SUPPLEMENTARY FIGURES

Figure S1 STRUCTURE bar graphs for ~~*k*=2, 3 and 4 for~~ 536 individuals of European sea bass showing membership to *k*=2, 3 and 4 ~~-~~clusters. Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each color a cluster. For full color representation consult the e-paper.

Comment [Filip Vol12]: REF 3: add sample codes

Figure S2 STRUCTURE bar graphs for ~~*k*=2, 3 and 4 for~~ 644 individuals of European sea bass showing membership to *k*=2, 3 and 4 ~~-~~clusters. Each vertical bar represents an individual genotyped at 46 SNP loci, and each color a cluster. For full color representation consult the e-paper.

Figure S3 STRUCTURE bar graphs for all individuals of European sea bass from the Atlantic Ocean showing membership to *k*=2 and 3 clusters genotyped at (a) 14 microsatellite (n = 214) and (b) 46 SNP loci (n=256). Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each color a cluster. For full color representation consult the e-paper.

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Figure S4 STRUCTURE bar graphs for all individuals of European sea bass from the Mediterranean Sea showing membership to *k*=2, 3 and 4 clusters genotyped at (a) 14 microsatellite (n = 322) and (b) 46 SNP loci (n = 392). Each vertical bar represents an individual genotyped at 14 microsatellite loci, and each color a cluster. For full color representation consult the e-paper.

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LIST OF TABLES

Table 1 Description of the samples of *Dicentrarchus labrax* collected, including geographic origin, sampling site, code, sampling date, sample size (*N*) of fish genotyped for SNPs, microsatellites and ~~combined~~ SNPs and microsatellites combined.

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Table 2 SNP and microsatellite loci detected under putative directional selection by the software packages LOSITAN and BAYESCAN in Atlantic, Mediterranean, Western Mediterranean and Eastern Mediterranean samples of European sea bass. Results from the LNRH tests are shown for microsatellites as the number of combinations in which loci were detected under selection (only numbers > 2 are reported). Loci indicated by * and ** were detected with a confidence interval of 95 % and 99.5 % for LOSITAN and 95% and 99% for BAYESCAN ~~ARE INDICATED BY * AND ** RESPECTIVELY~~. Results are listed as LNRH/LOSITAN/BAYESCAN for microsatellites and LOSITAN/BAYESCAN for SNPs. Outlier loci detected by at least two approaches are indicated in bold.

	Locus	Atlantic Ocean	Mediterranean Sea	Western Mediterranean Sea	Eastern Mediterranean Sea
Microsatellites	DLA0016	- / - / **	- / ** / -	~~- / * / -~~	
	DLA0119	**- / - / ** **	**2 / * * / -**	~~**- / * / -**~~	
	DLA0248		**7 / ** / -**		**2 / ** / -**
	DLA0110	4 / - / -	- / - / **		
	DLA0008	**6 / - / ** -**	**3 / - / ** -**	**- / - / ****	**2 / - / ** -**
	DLA0145	**- / - / ***	**3 / - / ** -**	**3 / - / -**	
	DLA0142	**- / - / ****	**5 / - / ** -**	**- / - / ****	**2 / - / -**
	DLA0146	**- / - / ****	**2 / - / ** -**	**2 / - / ** -**	
	DLA0140	- / - / *	- / - / **		
	DLA0020	- / - / **	- / - / *		
	DLA0105	- / - / **	- / - / **	- / - / **	
	DLA0228		- / - / *		
SNPs	~~CYP1~~	~~* / -~~			
	Dl_21p3	** / -			
	~~Dl_22l7~~		~~* / -~~		~~* / -~~
	Dl_26f8	** / -	** / -	** / -	
	Dl_32m8	** / -			~~* / -~~
	Dl_36d23	** / -			
	Dl_38e22	** / -			
	~~Dl_9f6~~				~~* / -~~
	ILB1-int2	** / -			
	SL-UTR1~~NPY-UTR~~	~~* / -~~	~~** / **~~		~~** / *~~
	SOX10~~NS13~~		~~** / -~~	~~* / -~~ ~~* / -~~	
	YY~~SL-UTR1~~		~~* / -~~ ~~** / **~~	~~** / -~~	~~** / *~~
	~~SOX10~~		~~** / -~~	~~* / -~~	
	~~YY~~		~~* / -~~	~~** / -~~	

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SUPPLEMENTARY INFORMATION

Table S1 Characteristics of the sequences containing microsatellites, including the name of the microsatellite, the functional annotation of the sequence, the linkage group of the sequence in the sea bass genome, the repeat motif, the number of alleles and the GenBank accession number

Microsatellite name	Sequence annotation	Sea bass linkage group	Repeat motif	N alleles	GenBank accession number
DLA0008	-	LG 24	(ac)24	57	AY221746
DLA0016	-	LG 1	(tg)24	28	AY262073
DLA0020	-	LG 12	(tg)20	19	AY262077
DLA0105	-	LG 8	(ac)16	28	AY262082
DLA0110	-	LG 25	(gt)17	22	AY262087
DLA0119	-	LG 14	(tg)10	26	AY302259
DLA0140	-	LG 15	(ac)30	23	AY430370
DLA0142	Ephrin receptor fragment	LG 15	(ac)30	31	AY430372
DLA0145	-	LG 17	(tc)20	22	AY430375
DLA0146	-	LG 10	(tg)27	38	AY430376
DLA0228E	-	LG 10	(aaag)3(ag)4(aaag)3	14	AY639896
DLA0237PY	mRNAfor Peptide Y	LG 1	(tc)4(c)2(tc)2(c)2(tc)10	9	AJ005380
DLA0244	-	LG 10	(tg)12(ag)5(tg)2	11	AY714328
DLA0248	-	LG 15	(tc)5(ac)(at)(tc)5(t)2(tc)7(ac)3(acgc)4	9	AY714332

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Table S2 Characterisation of the sequences containing SNPs by the procedure of development, including the name of the SNP, the functional annotation of the sequence, the SNP annotation (S: synonymous; NS: non-synonymous), the location of the sequence in the sea bass genome (linkage group number), the frequency of the minor allele (MAF) and the GenBank accession number. Loci that were discarded from the analysis because of non-Mendelian inheritance or putative presence of null alleles are indicated by *.

SNP development	SNP name	Sequence annotation	Sea bass linkage group	SNP location	MAF	GenBank accession number
ESTs, Atlantic samples	S25	-	LG13	-	0.226	JM497213
	S29	cathepsin C precursor	LG13	-	0.364	JM497214
	S30	cornifelin	LG16	-	0.105	JM497216
	S66	stress-induced-phosphoprotein 1	LG14	CDS (S)	0.432	JM497229
	S79	-	LG5	-	0.167	JM497236
	S98	-	LGx	-	0.204	JM497249
	S106	Ferritin--livermiddlesubunit	LG8	CDS (S)	0.025	JM497160
	S110*	ATP synthase -- H+ transporting -- mitochondrial Fo complex -- subunit B1	LG22-25	CDS (S)	0.196	JM497164
	S115	MOB1 -- Mps One Binder kinase activator-like 2A	LG4	CDS (S)	0.469	JM497167
	S118	zincfinger, DHHC-type containing 4	LG1B	intronic	0.333	JM497168
	S131	UPF0561 protein C2orf68 homolog	LG20	?	0.274	JM497176
	S159	glutathione S-transferasealpha 5	LG11	CDS (S)	0.487	JM497181
ESTs, Mediterranean and Atlantic samples	S9	Hemoglobin subunit beta-1 (Hemoglobin beta-1 chain)(Beta-1-globin)(Beta-A1-globin)	SB	intronic	0.004	JM497243
	S78	potassium intermediate/small conductance calcium-activated channel -- subfamily N -- member 4	LG16	?	0.173	JM497235
	S89	RCD1 required for cell differentiation1 homolog (S. Pombe)	LG15	intronic	0.134	JM497242
	S90	Macrophage mannose receptor 1	LG9	intronic	0.442	JM497244
	S91	SUB1 homolog (S. Cerevisiae)	LG20	intronic	0.061	JM497245
	S109	Obg-likeATPase 1 (EC 3.6.3.-)	LG12	CDS (S)	0.043	JM497162
	S119	Sin3A-associated protein -- 18kDa	LG24	CDS (S)	0.208	JM497169
	S148	Uncharacterizedprotein C8orf59 homolog	LG18	UTR	0.052	JM497180
	S170*	Uncharacterizedprotein	LG1A	CDS	0.272	JM497186
	S181	superoxide dismutase 1 – soluble	LG14	UTR	0.165	JM497190
	S186	protein tyrosine phosphatase -- non-receptor type 9	LG6	intronic	0.013	JM497192
	S218*	Hemoglobin subunit alpha (Hemoglobin alpha chain)(Alpha-globin aa1)	SB	CDS (S)	0.215	JM497211
	NS13	-	LG20	-	0.210	JM497135
	NS35	fructosamine 3 kinase	LG8	intronic	0.005	JM497148
	NS36	Keratin--type I cytoskeletal 18	LG22-25	UTR	0.247	JM497149
ESTs, Mediterranea	SL-UTR1	somatolactin	LG13	UTR	0.141	AJ277390
	ERB-UTR	estrogen receptor 2 (ER beta)	LG17	UTR	0.294	AJ489523

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	n samples	MT-int1	Metallothionein (MT)	LG6	CDS (NS)	0.403	AF199014
		RAG-ex2	recombinationactivating gene 1	LG6	intronic	0.451	AF369066
		ACB-UTR	Beta-actin (Fragment)	LG8	UTR	0.168	AJ537421
		SOX10	SRY (sex determining region Y)-box 10	LG7	intronic	0.202	AY247003
		NPY-UTR	neuropeptide Y	LG9	UTR	0.096	AJ005378
		ILB1-int2	interleukin 1 -- beta	LG20	intronic	0.385	AJ311925
		CYP1	cytochrome P450 -- family 1 -- subfamily A -- polypeptide 2	LG5	UTR	0.295	AJ251913
		YY	Peptide YY-like	LG16	CDS (S)	0.202	AJ005379
	BAC end sequences, Mediterranean samples	Dl_4h3	-	LG9	-	0.151	
		Dl_13f21	LIM domain binding 2	LG2	intronic	0.119	
		Dl_21p3	-	LG6	-	0.331	
		Dl_13o18	-	LG17	-	0.179	
		Dl_9f6	phospholipase B1	LG12	?	0.338	
		Dl_26f8	ankyrin repeat and SOCS box containing 5	LG2	intronic	0.440	
		Dl_32m8	-	LG7	-	0.479	
		Dl_22l7	-	LG20	-	0.196	
		Dl_36d23	ADAM metallopeptidase with thrombospondin type 1 motif -- 16	LG16	intronic	0.410	
		Dl_6k11	protein tyrosine phosphatase -- receptor type -- S	LG4	intronic	0.002	
		Dl_37g3	gem (nuclear organelle) associated protein 5	LG14	intronic	0.338	
		Dl_38e22	O-6-methylguanine-DNA methyltransferase	LG11	intronic	0.421	

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Table S3 Genetic variability and multi-locus F_{IS} estimates at 14 microsatellites of *Dicentrarchus labrax*. A = Average number of alleles per locus; H_{exp} = unbiased expected heterozygosity; H_{obs} = observed heterozygosity; F_{IS} = multi-locus F_{IS} estimate (significant values are listed before and after Bonferroni correction underlined and in bold respectively)

Sample	A	H_{exp}	H_{obs}	F_{IS}
NOR	15.00	0.807 (0.17)	0.788 (0.19)	0.023
IRW	10.93	0.804 (0.18)	0.786 (0.18)	0.023
IRC	12.57	0.815 (0.17)	0.793 (0.18)	0.028
BEL	16.29	0.819 (0.16)	0.817 (0.17)	0.003
FSML	11.64	0.790 (0.18)	0.822 (0.17)	-0.043
POR	11.14	0.789 (0.19)	0.701 (0.14)	**0.115**
PFA	9.36	0.825 (0.13)	0.786 (0.18)	0.050
MRBT	12.00	0.793 (0.17)	0.732 (0.18)	0.079
MKS1	11.86	0.822 (0.13)	0.783 (0.13)	0.049
EMUR	13.29	0.780 (0.14)	0.780 (0.13)	-0.001
EGLV	9.21	0.765 (0.15)	0.707 (0.13)	0.079
FSET	13.07	0.794 (0.13)	0.784 (0.13)	0.014
MUR	10.43	0.767 (0.15)	0.688 (0.16)	0.110
FCT	7.71	0.755 (0.18)	0.755 (0.17)	0.000
SCL	9.36	0.799 (0.13)	0.766 (0.19)	0.042
IVEN	11.64	0.773 (0.14)	0.760 (0.11)	0.016
IPT	11.57	0.763 (0.13)	0.738 (0.09)	0.033
CMT	8.21	0.775 (0.14)	0.821 (0.20)	-0.063
GMES	10.79	0.762 (0.15)	0.769 (0.14)	-0.009
GCRE	6.00	0.768 (0.14)	0.818 (0.14)	-0.068
EGL	7.50	0.717 (0.21)	0.707 (0.20)	0.014
Atlantic Ocean	22.07	0.814 (0.16)	0.785 (0.14)	**0.036**
Mediterr. Sea	19.93	0.781 (0.14)	0.757 (0.10)	0.016
West Mediterr.	18.50	0.787 (0.14)	0.756 (0.11)	**0.04**
East Mediterr.	16.43	0.773 (0.14)	0.758 (0.11)	0.02

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Table S4 Genetic variability and multi-locus F_{IS} estimates at 46 SNPs of *Dicentrarchus labrax*. A = Average number of alleles per locus; H_{exp} = unbiased expected heterozygosity; H_{obs} = observed heterozygosity; F_{IS} = multi-locus F_{IS} estimate. Significant values before and after Bonferroni correction are listed underlined and in bold respectively.

Sample	A	H_{exp}	H_{obs}	F_{IS}
NOR	1.80	0.221 (0.20)	0.227 (0.21)	-0.029
IRW	1.76	0.229 (0.21)	0.205 (0.19)	0.106
IRC	1.72	0.216 (0.21)	0.205 (0.21)	0.052
BEL	1.83	0.224 (0.20)	0.219 (0.20)	0.024
FSML	1.87	0.224 (0.19)	0.228 (0.20)	-0.020
POR	1.72	0.221 (0.19)	0.236 (0.22)	-0.071
PFA	1.83	0.270 (0.19)	0.279 (0.22)	-0.038
MRBT	1.87	0.255 (0.18)	0.257 (0.19)	-0.009
MKS1	1.87	0.248 (0.19)	0.231 (0.18)	0.072
EMUR	1.96	0.288 (0.17)	0.287 (0.18)	0.003
EGLV	1.93	0.288 (0.17)	0.261 (0.16)	0.093
FSET	1.89	0.292 (0.18)	0.290 (0.18)	0.009
MUR	1.87	0.289 (0.17)	0.304 (0.19)	-0.056
FCT	1.87	0.288 (0.17)	0.278 (0.19)	0.037
SCL	1.87	0.292 (0.17)	0.290 (0.18)	0.006
IVEN	1.91	0.272 (0.19)	0.263 (0.18)	0.034
IPT	1.91	0.283 (0.18)	0.268 (0.18)	0.056
CMT	1.91	0.324 (0.18)	0.283 (0.18)	0.132
GMES	1.85	0.283 (0.18)	0.286 (0.19)	-0.010
GCRE	1.67	0.254 (0.21)	0.273 (0.26)	-0.082
GTSK	1.89	0.239 (0.17)	0.235 (0.19)	0.019
EGL	1.70	0.217 (0.20)	0.204 (0.19)	0.062
Atlantic Ocean	1.98	0.233 (0.18)	0.229 (0.18)	0.01
Mediterr. Sea	1.98	0.287 (0.17)	0.272 (0.16)	0.023
West Mediterr.	1.98	0.290 (0.17)	0.285 (0.16)	0.02
East Mediterr.	1.93	0.278 (0.18)	0.259 (0.17)	**0.06**

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Table S5 Matrix of pair-wise estimates of F_{ST} (Θ) using all 14 microsatellites of *Dicentrarchus labrax* and sample sites with at least 15 individuals. Significant values before and after sequential Bonferroni correction are indicated underlined and bold respectively. Atlantic and Alboran samples are indicated in italics. For sample codes see Table 1

	IRW	*IRC*	*BEL*	*FSML*	*POR*	*MRBT*	*MKS1*	EMUR	EGLV	FSET	MUR	FCT	SCL	IVEN	IPT	GMES
NOR	0.	0.	0.	0.	0.0037	0.0121	0.0055	**0.0607**	**0.0738**	**0.0622**	**0.0663**	**0.0791**	**0.0559**	**0.0773**	**0.0809**	**0.0865**
IRW		0.	0.	0.	0.0009	0.0134	0.0030	**0.0548**	**0.0609**	**0.0594**	**0.0608**	**0.0705**	**0.0502**	**0.0728**	**0.0737**	**0.0841**
IRC			0.0013	0.0022	0.0044	0.0182	0.0035	**0.0564**	**0.0666**	**0.0579**	**0.0629**	**0.0807**	**0.0481**	**0.0723**	**0.0739**	**0.0807**
BEL				0.0014	0.0064	0.0093	0.0076	**0.0439**	**0.0552**	**0.0451**	**0.0508**	**0.0595**	**0.0385**	**0.056**	**0.0621**	**0.068**
FSML					0.0017	0.0124	0.0043	**0.0696**	**0.0809**	**0.066**	**0.0749**	**0.0937**	**0.0651**	**0.084**	**0.089**	**0.0989**
POR						0.0084	0.0024	**0.0688**	**0.0737**	**0.0642**	**0.0698**	**0.086**	**0.0562**	**0.0763**	**0.0827**	**0.0908**
MRBT							**0.0172**	**0.0561**	**0.0666**	**0.0587**	**0.0576**	**0.0712**	**0.0492**	**0.0621**	**0.0763**	**0.0847**
MKS1								**0.0485**	**0.0486**	**0.0457**	**0.0457**	**0.0646**	**0.0372**	**0.0582**	**0.0547**	**0.0676**
EMUR									**0.017**	0.0009	0.0048	**0.0208**	**0.0152**	**0.0127**	**0.0125**	0.01
EGLV										0.0165	0.0039	0.0245	0.0106	**0.016**	0.0068	0.0161
FSET											0.0065	**0.0297**	**0.0206**	**0.0148**	**0.0179**	**0.0137**
MUR												0.0094	0.0039	0.0082	0.0049	0.0085
FCT													0.0187	**0.0255**	**0.0191**	**0.0295**
SCL														0.0111	0.0113	**0.0202**
IVEN															**0.011**	**0.0154**
IPT																**0.0101**

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Table S6 Results of the Mantel tests for evidence of isolation by distance for microsatellite and SNP loci of European sea bass, including partial Mantel tests when controlling for latitude, longitude, geographic and genetic distances. *Z*: Mantel coefficient; *r*: correlation index; *p*: significance level (significant values are listed in bold)

			Atlantic	Mediterranean	Western Mediterranean	Eastern Mediterranean
Microsatellites	Genetic distances vs Geographic distances	*Z*	196,260	1,710,752	165,783	283,369
		r	0.379	0.509	0.167	0.899
		p	**0.038**	**0.042**	0.211	**0.039**
	Genetic distances vs Geographic distances, controlling for latitude	*r*	0.371	0.264	0.174	0.866
		p	**0.045**	0.052	0.207	0.204
	Genetic distances vs Geographic distances, controlling for longitude	*r*	0.388	0.047	-0.310	0.494
		p	**0.025**	0.370	0.233	0.332
	Genetic distances vs latitude	*Z*	1.718	5.621	0.570	1.538
		r	0.559	0.561	0.071	0.839
		p	**0.002**	0.057	0.408	**0.042**
	Genetic distances vs latitude, controlling for genetic distances	*r*	0.555	0.373	0.086	-0.782
		p	**0.036**	0.063	0.389	0.204
	Genetic distances vs longitude	*Z*	0.474	13.464	1.616	2.241
		r	-0.119	0.646	0.303	0.892
		p	0.405	**0.029**	0.084	**0.039**
	Genetic distances vs longitude, controlling for genetic distances	*r*	0.150	0.463	0.395	-0.439
		p	0.155	**0.037**	0.149	0.332
SNPs	Genetic distances vs	*Z*	653,598	4,309,638	65,775	830,888

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Geographic distances	*r*	0.683	0.643	-0.221	0.815
	p	**0.000**	**0.001**	0.143	**0.017**
Genetic distances vs Geographic distances, controlling for latitude	*r*	-0.156	0.536	-0.226	0.713
	p	0.246	**0.001**	0.147	**0.034**
Genetic distances vs Geographic distances, controlling for longitude	*r*	0.799	0.541	-0.128	0.478
	p	**0.003**	**0.010**	0.409	0.115
Genetic distances vs latitude	*Z*	4.995	12.130	0.251	3.839
	r	0.736	0.485	-0.043	0.603
	p	**0.001**	**0.015**	0.463	**0.048**
Genetic distances vs latitude, controlling for genetic distances	*r*	0.405	0.265	-0.064	-0.264
	p	**0.018**	0.114	0.443	0.271
Genetic distances vs longitude	*Z*	1.615	29.477	0.599	5.806
	r	-0.075	0.432	-0.188	0.751
	p	0.465	**0.028**	0.147	**0.017**
Genetic distances vs longitude, controlling for genetic distances	*r*	-0.571	-0.136	0.045	-0.040
	p	**0.018**	0.265	0.472	0.481

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Table S7 Matrix of pair-wise estimates of F_{ST} (Θ) using all the 46 SNPs of *Dicentrarchus labrax* and sample sites with at least 15 individuals. Significant values before and after sequential Bonferroni correction are indicated underlined and bold respectively. Atlantic and Alboran samples are indicated in italics. For sample codes see Table 1

	IRW	*IRC*	*BEL*	*FSML*	*POR*	*MRBT*	*MKS*	EMUR	EGLV	FSET	MUR	FCT	SCL	IVEN	IPT	GMES	GTSK	EGL
NOR	0.	0.0036	0.0047	0.	0.0057	**0.021**	**0.0271**	**0.2753**	**0.2944**	**0.3138**	**0.3123**	**0.2842**	**0.3003**	**0.3543**	**0.385**	**0.3617**	**0.4297**	**0.4443**
IRW		0.	0.	0.	0.0031	0.0213	0.0228	**0.2626**	**0.2824**	**0.3005**	**0.2942**	**0.2709**	**0.2849**	**0.3379**	**0.371**	**0.3499**	**0.4216**	**0.4391**
IRC			0.0024	0.0067	0.0022	0.0223	0.0211	**0.2636**	**0.2818**	**0.2988**	**0.2951**	**0.273**	**0.2874**	**0.3377**	**0.3707**	**0.3489**	**0.4206**	**0.4369**
BEL				0.0024	0.0014	**0.0265**	**0.0236**	**0.2765**	**0.2966**	**0.3157**	**0.3109**	**0.2837**	**0.302**	**0.351**	**0.3846**	**0.3627**	**0.4298**	**0.4415**
FSML					0.0045	0.0143	**0.026**	**0.2654**	**0.287**	**0.3064**	**0.3007**	**0.2769**	**0.2962**	**0.342**	**0.3776**	**0.3539**	**0.4173**	**0.4281**
POR						0.0154	0.0096	**0.2424**	**0.2627**	**0.2818**	**0.2769**	**0.2499**	**0.2713**	**0.3247**	**0.3568**	**0.3319**	**0.409**	**0.421**
MRBT							0.0087	**0.1699**	**0.1881**	**0.2116**	**0.2059**	**0.1753**	**0.1968**	**0.2457**	**0.2855**	**0.2585**	**0.3187**	**0.3304**
MKS1								**0.1903**	**0.2034**	**0.2242**	**0.2204**	**0.1982**	**0.2113**	**0.2675**	**0.2999**	**0.2761**	**0.345**	**0.3595**
EMUR									0.0007	0.0044	0.0039	0.	0.0172	**0.0346**	**0.0723**	**0.0327**	**0.0647**	**0.0794**
EGLV										0.	0.0015	0.0008	0.0048	**0.0209**	**0.0481**	**0.0183**	**0.0456**	**0.0566**
FSET											0.0009	0.0072	0.0127	**0.0206**	**0.0438**	**0.0166**	**0.0429**	**0.0479**
MUR												0.0088	0.0125	**0.0286**	**0.0648**	**0.0182**	**0.0434**	**0.0656**
FCT													0.0193	**0.0284**	**0.0561**	**0.0329**	**0.0761**	**0.0889**
SCL														**0.0409**	**0.068**	**0.0383**	**0.0699**	**0.0944**
IVEN															0.0173	**0.0242**	**0.05**	**0.0519**
IPT																**0.0403**	**0.0744**	**0.0758**
GMES																	0.014	**0.0519**
GTSK																		**0.0403**

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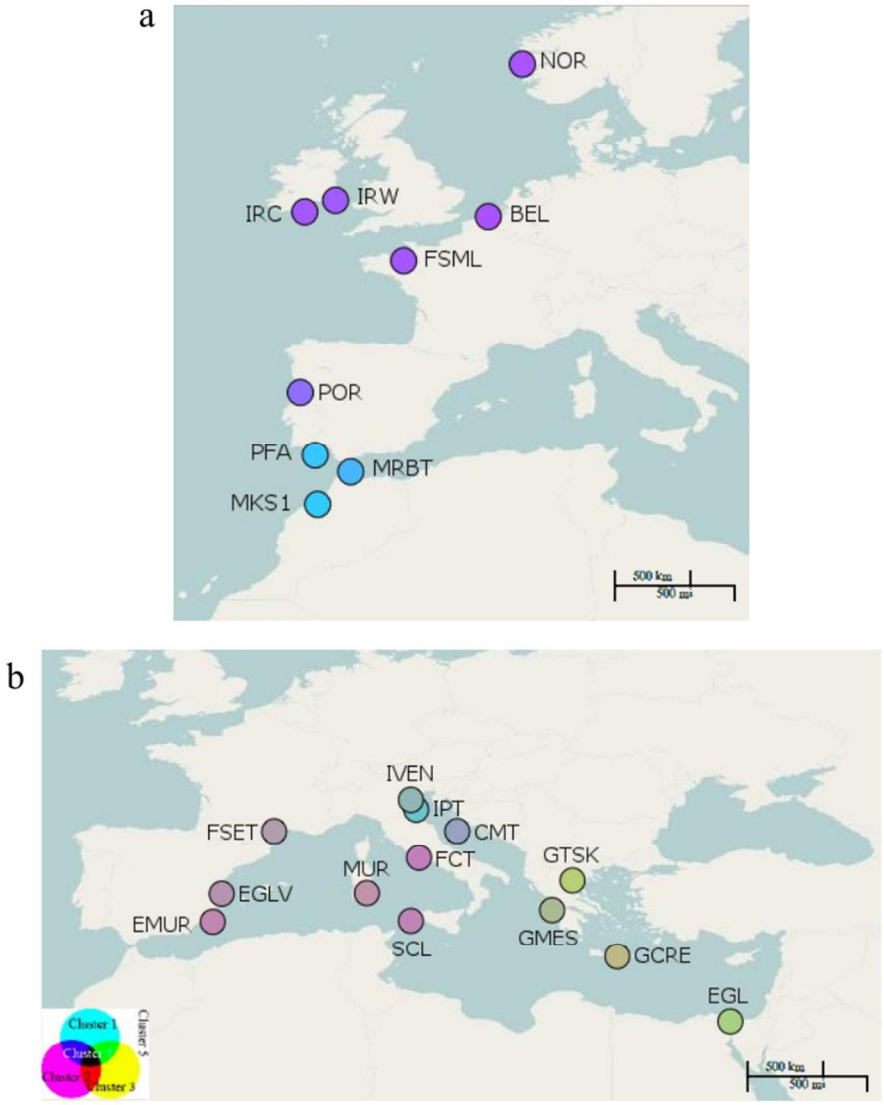
Table 1 Description of the samples of *Dicentrarchus labrax* collected, including geographic origin, sampling site, code, sampling date, sample size (*N*) of fish genotyped for SNPs, microsatellites and combined SNPs and microsatellites

Geographic origin	Sampling site	Code	Longitude	Latitude	Sampling date	Sampling contact - source publication	*N* SNPs	N Micro-satellites	N SNPs & mi-crosatellites
Atlantic Ocean	Masfjord, Norway	NOR	5°20'E	60°49'N	2005	J. Holmen	40	34	34
	Wexford, Ireland	IRW	6°26'W	52°20'N	2007	S. Mariani	21	16	16
	Cork, Ireland	IRC	8°15'W	51°50'N	2006-2007	S. Mariani	25	23	22
	Zeebrugge, Belgium	BEL	3°11'E	51°21'N	2007	E. Cuveliers	42	40	40
	Saint-Malo, France	FSML	2°08'W	48°46'N	2002	B. Chatain	41	23	23
	Aveiro, Portugal	POR	8°42'W	40°39'N	1993	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Naciri *et al.* (1999); Allegrucci *et al.* (1999)	21	19	19
	Faro, Portugal	PFA	7°51'W	36°53'N	2008	A. Canario	12	12	12
	Rabat, Morocco	MRBT	7°36'W	33°53'N	1996	Naciri *et al.* (1999); Bonhomme *et al.* (2002); Lemaire *et al.* (2005)	31	24	24
	Ksar es Seghir, Morocco	MKS1	5°33'W	35°51'N	1997	Naciri *et al*. (1999); Lemaire *et al.* (2005)	23	23	23
Western Mediterranean Sea	Murcia, Spain	EMUR	0°47'W	37°44'N	2004	Quéré *et al.* (2012)	40	40	40
	Valencia, Spain	EGLV	0°10'E	39°22'N	1994	García de Léon *et al.* (1997); Naciri *et al.* (1999); Bahri-Sfar *et al.* (2000)	36	18	17
	Sète, France	FSET	3°45'E	43°22'N	2006	Quéré *et al.* (2012)	40	36	36
	Muravera, Italy	MUR	9°37'E	39°26'N	1992	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Cesaroni *et al.* (1997);	33	29	29

						Allegrucci *et al.* (1999); Lemaire *et al.* (2000)			
	Fiumicino, Italy	FCT	12°70'E	41°51'N	1990	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Cesaroni *et al.* (1997); Allegrucci *et al.* (1999); Lemaire *et al.* (2000); Quéré *et al*. (2012)	21	16	15
	Marsala, Italy	SCL	12°18'E	37°50'N	1991	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Cesaroni *et al.* (1997); Naciri *et al.* (1999); Allegrucci *et al.* (1999); Lemaire *et al.* (2000); Bahri-Sfar *et al*. (2000); Quéré *et al.* (2012)	21	19	18
Eastern Mediterranean Sea	Venice, Italy	IVEN	12°18'E	45°20'N	2005	L. Bargelloni	39	43	39
	Porto Tolle, Italy	IPT	12°52'E	44°59'N	2000-2005	L. Bargelloni	41	40	40
	Murter, Croatia	CMT	15°14'E	43°23'N	2006	T. Patarnello	11	12	11
	Messolongi, Greece	GMES	21°20'E	38°18'N	2005	F. Bonhomme	45	39	39
	Crete, Greece	GCRE	25°36'E	35°20'N	2006	F. Bonhomme	6	9	6
	Thessaloniki, Greece	GTSK	22°50'E	40°06'N	1997	Bahri-Sfar *et al*. (2000); Bonhomme *et al.* (2002)	31	-	-
	Bardawil, Egypt	EGL	32°58'E	31°07'N	1991	Allegrucci *et al.* (1997); Caccone *et al.* (1997); Cesaroni *et al.* (1997); Allegrucci *et al.* (1999); Lemaire *et al.* (2000); Bahri-Sfar *et al.* (2000)	24	21	21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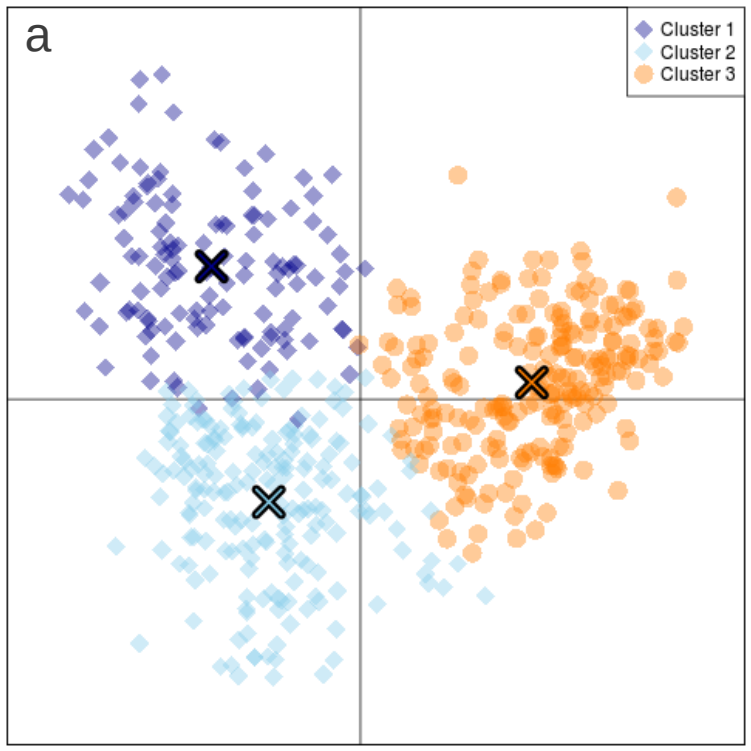
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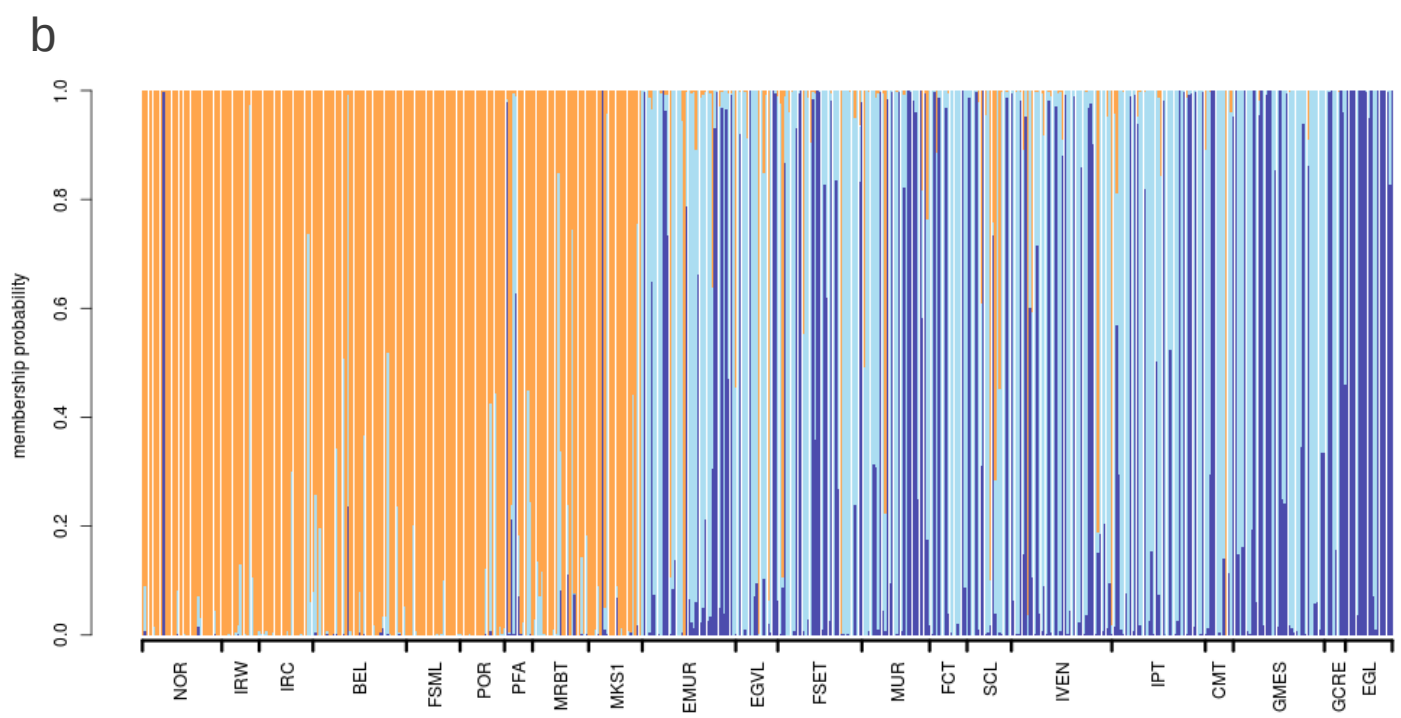
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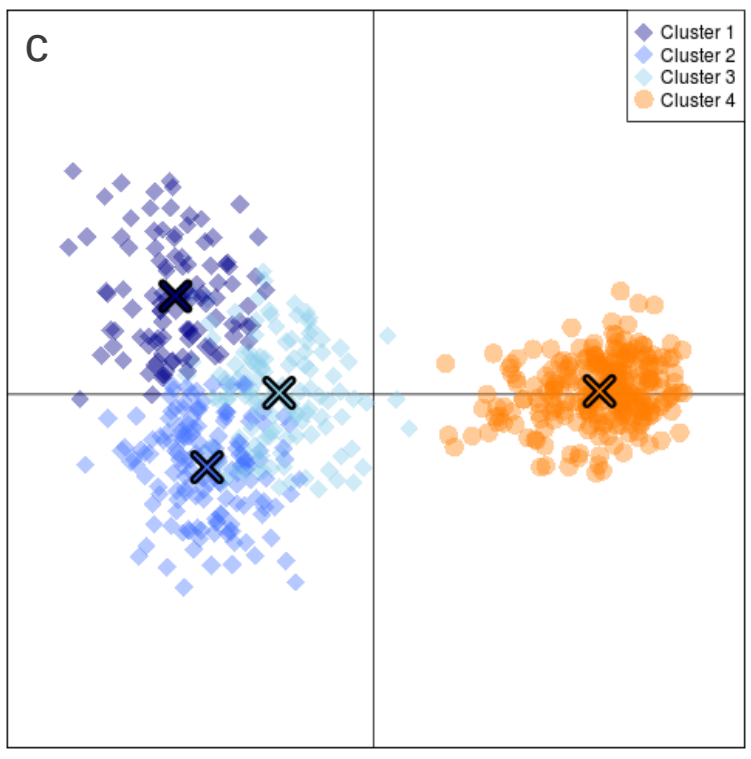
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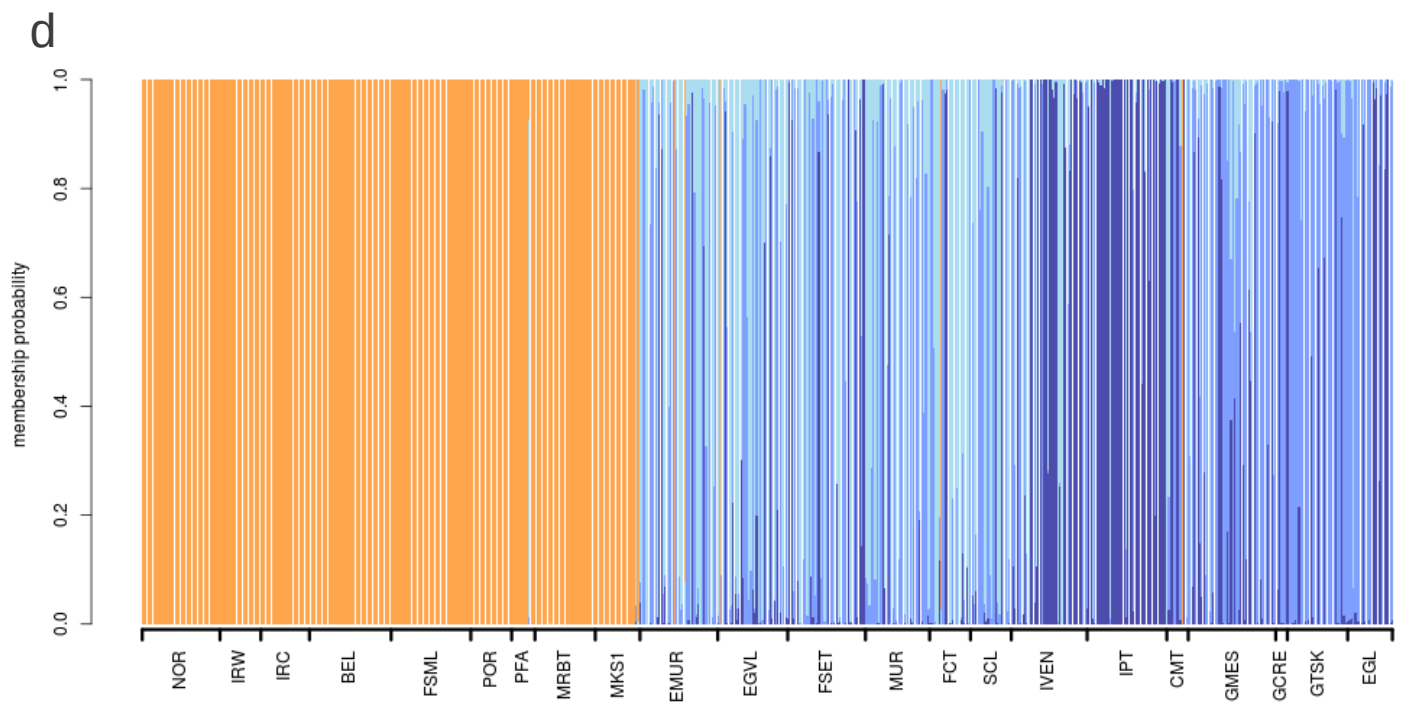
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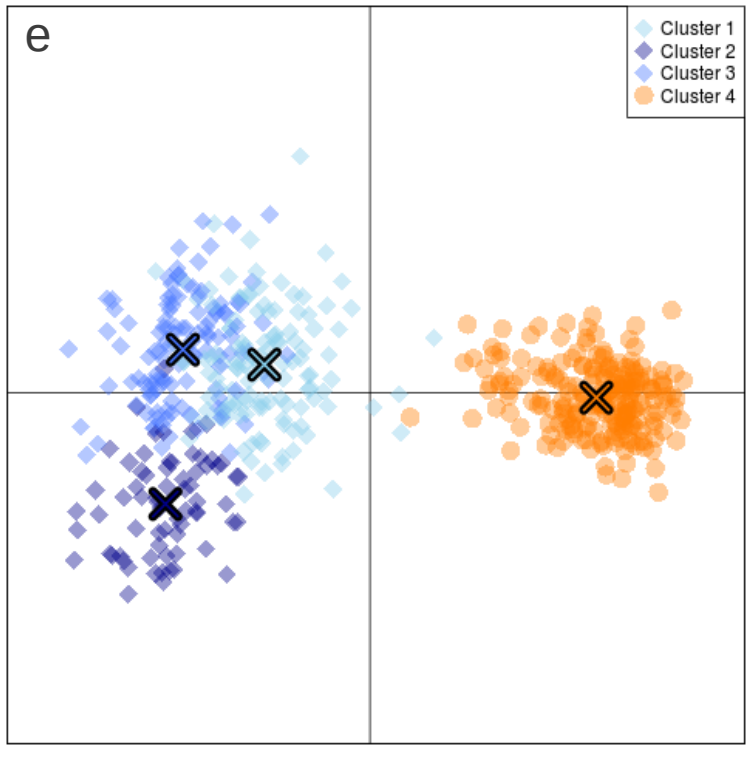
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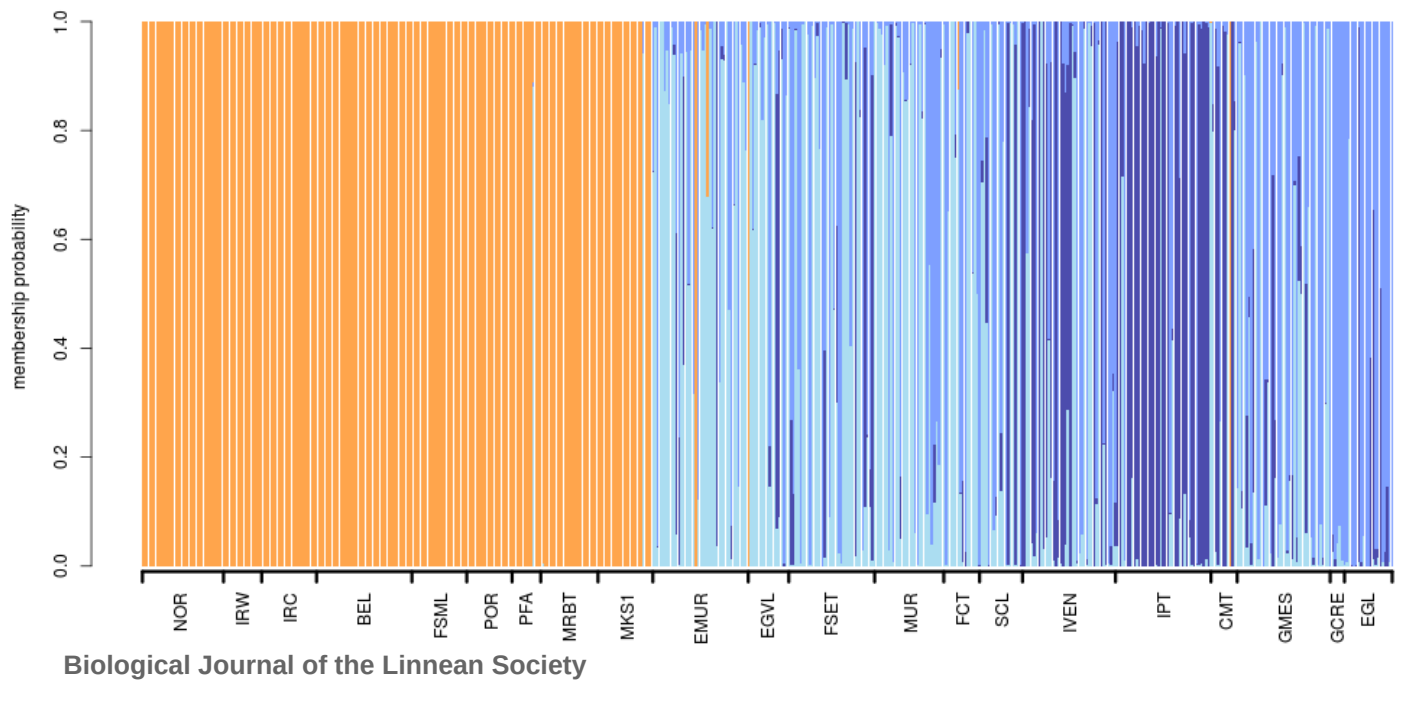
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MKS1
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EGVL
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GMES
GCRE
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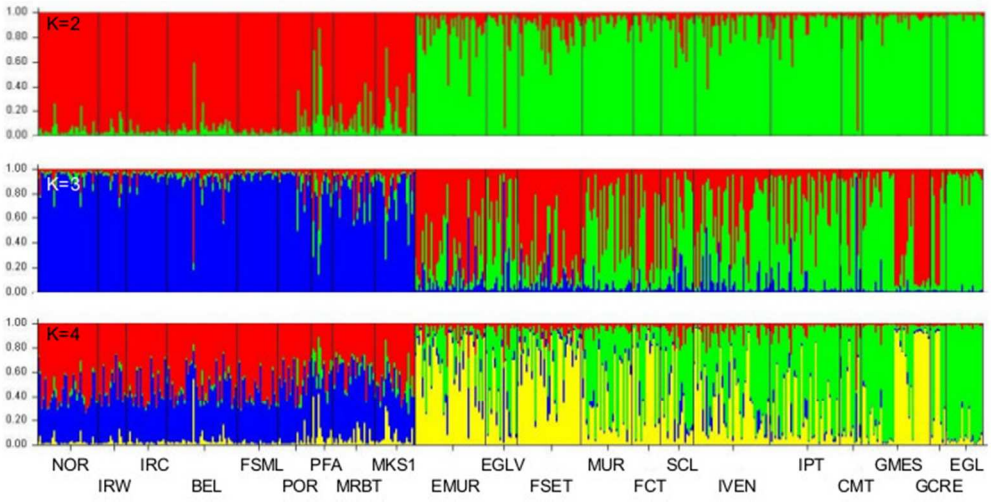

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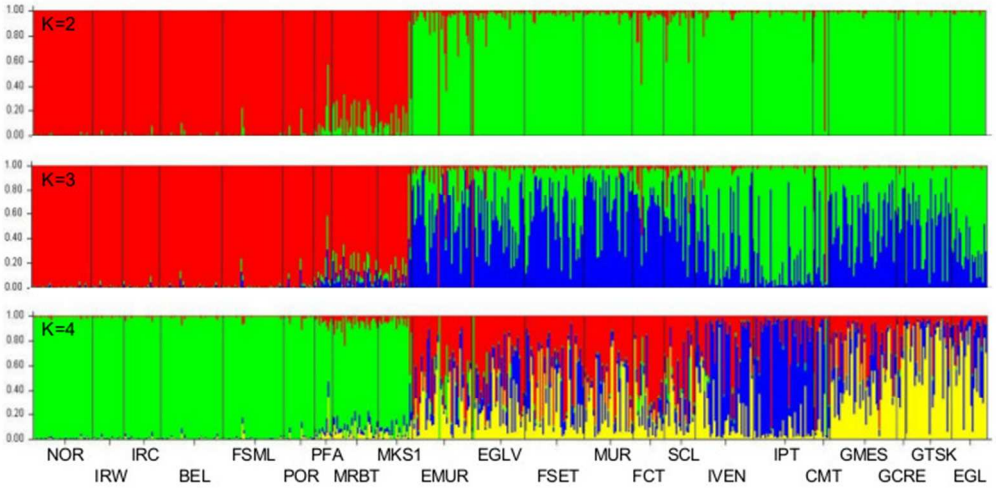
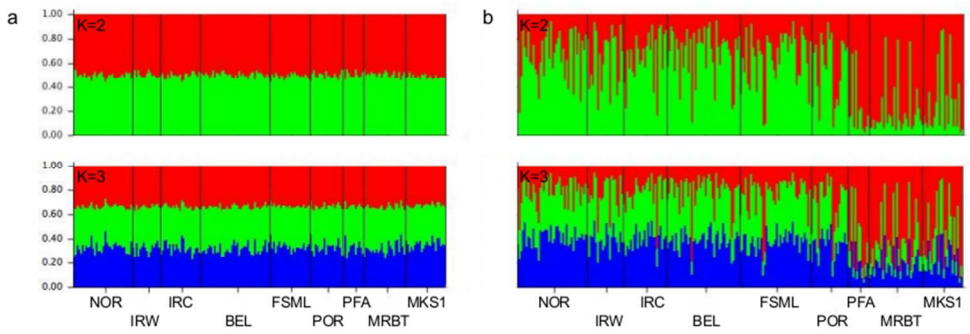
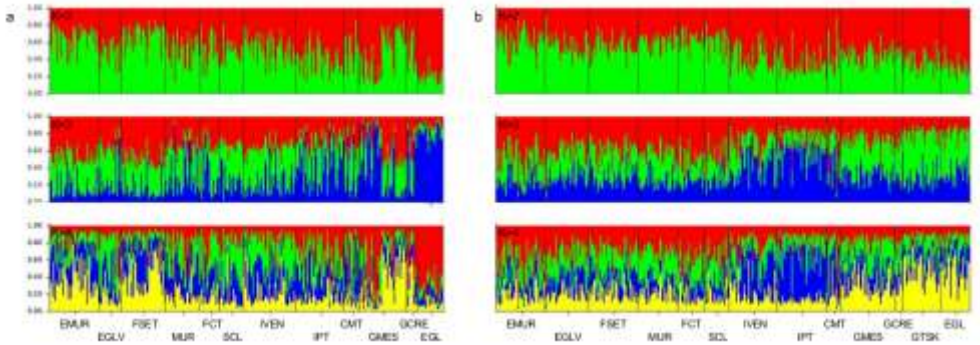


192x97mm (100 x 100 DPI)



219x78mm (100 x 100 DPI)



311x115mm (100 x 100 DPI)